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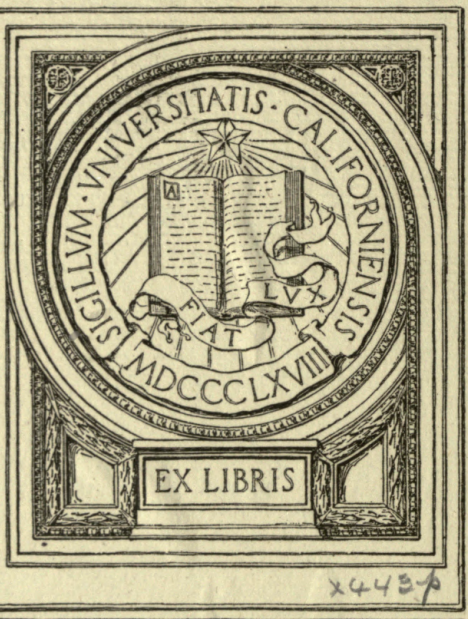

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ABSCISSION OF FLOWERS AND FRUITS IN THE SOLANACEAE, WITH SPECIAL REFERENCE TO *NICOTIANA*

A THESIS SUBMITTED IN PARTIAL SATISFACTION OF
THE REQUIREMENTS FOR THE DEGREE OF
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AT THE UNIVERSITY OF CALIFORNIA

BY

JOHN NORMAN KENDALL

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INTRODUCTION

Although it is a matter of common observation that many plants are capable of detaching portions of the body, the underlying cause and the actual mechanism which bring about such separation are only slightly understood. The process has often been described as one of self-pruning by which the plant rids itself of useless portions of its body. Since abscission is sometimes confused with exfoliation, it seems desirable here to distinguish definitely between these two phenomena. It can be said that, in general, exfoliation is preceded by drying and death of the part to be cast off and that actual separation of the organ is accomplished by a mechanical break through dry, dead tissues. Abscission, on the other hand, is usually not preceded by drying and death of the organ concerned and its detachment is accomplished by a separation along the plane of the middle lamellae of active living cells.

Abscission may be either axial or lateral. Axial abscission includes the abscission of portions of stems, shoots, entire flowers or fruits. Lateral abscission includes the abscission of leaves, petioles, sepals, petals or styles. Considerable attention has been given by investigators to the abscission of flowers because of the theoretical detriment to crops caused by the fall of the flower before the fruit is formed.

The cause of leaf-fall in deciduous species is connected with periodic changes in the physiological condition brought about by changes in the environment. In the case of some herbaceous plants and occasionally in trees, sudden changes in environmental conditions resulting in a loss of physiological equilibrium often cause the throwing off of leaves, flowers or even small shoots. In certain species, anything which tends to loss or completion of function within or peculiar to an organ causes the organ to be thrown off. Thus, staminate flowers are commonly thrown off soon after anthesis and pistilate flowers generally fall when fertilization is prevented. Similarly, certain species—e.g., *Impatiens Sultani* and *Mirabilis Jalapa*—throw off portions of their stems which have been rendered useless as a part of the conducting system because of injury or removal of distal buds or leaves.

The following definitions of terms, which will be used throughout this paper, are made necessary because of a notable lack of uniformity in their usage by various investigators who have dealt with abscission.

1. *Abscission* is the detaching of an organ by the separation of actively living cells at or near its base.

2. The *separation layer* (Mohl's *Trennungschichte*) is the layer of cells the components of which will separate from one another at abscission.

3. The *separation cells* or *absciss cells* are the cells that make up the separation layer.

4. The *separation zone* is the general region through which abscission takes place and usually is largely proximal to the separation layer.

A preliminary account of abscission in F_1 species hybrids of *Nicotiana* has already appeared (Goodspeed and Kendall, 1916). The present study represents an amplification of this investigation and its extension to other species of the Solanaceae. It is particularly concerned with the following: (1) the position of the separation layer; (2) the origin of the separation layer; (3) the cytology of the separation layer; (4) the process of abscission, including (*a*) a description of the appearance of the separation layer in consecutive stages of the process and (*b*) the method of cell separation; (5) the time occupied by abscission, including (*a*) the time between the application of the stimulus and fall (reaction period) and (*b*) the time involved in the actual process of cell separation (abscission period); (6) experimental induction of abscission.

Although the investigation reported here is largely a morphological one, the results of the experiments on the method of cell separation, the time of abscission and the induction of abscission seem to have a distinct physiological significance as well.

SUMMARY OF THE LITERATURE

Since the literature on abscission is rather voluminous, it seems best to present the following discussion under several different headings corresponding, to a certain extent, with the six main topics of interest mentioned in the introduction. The summary below is largely confined to the literature on axial abscission, although that on lateral abscission is considered in so far as it has a direct bearing on the most important aspects of the abscission problem.

1. HISTOLOGY OF THE PEDICEL

a. POSITION OF THE SEPARATION LAYER

Hoehnel (1880), discussing the fall of catkins in *Populus* and *Salix,* locates the separation layer at the base of the catkin. The general region at the base of the catkin, in the distal part of which the separation layer is located, he calls the "separation zone." In *Salix,* actual separation occurs in the separation layer, but in *Populus* it occurs in the parenchyma entirely outside the separation layer. According to Balls (1911), the separation layer in the cotton flower is located at the base of the pedicel. The layer is located by Hannig (1913) at the base of the pedicel in *Nicotiana Tabacum, N. rustica, N. accuminata, N. sylvestris, Datura,* and *Atropa,* and at the tip of the pedicel in *Nicotiana Langsdorffii, Salvia Aloe, Cuphea,* and *Gasteria.* He finds it occurring at the middle of the pedicel in *Impatiens Sultani, Solanum tuberosum, Lycopersicum, Asparagus,* and *Begonia.* Gortner and Harris (1914) and Lloyd (1914*b*), working on the abscission of internodes as the result of injury in *Impatiens Sultani,* locate the separation layer at the first node below the injury and just above the axillary bud. Occasionally, according to the latter investigators, abscission may occur at the second or third node below the injury and in these cases the buds at the first or second nodes seem to be abortive.

The separation layer, according to Hannig (1913), may occur at the base of the complete inflorescence in *Impatiens* and *Oxybaphus.* According to Lloyd (1914*a*), the separation layer occurs at the base of the pedicel in cotton and at the base of the ripened ovary in grape "shelling." In the abscission of internodes and tendrils in *Vitis* and *Ampelopsis,* Lloyd (1914*a*) locates the layer near but not exactly at the base of the internode. A peculiar case illustrating the result of displacement of the stem on the location of the separation layer is

discussed by Lloyd (1914*a*) for *Ampelopsis* and *Gossypium*. In the latter, abscission, in the abnormal case, occurred down the internode at the base of the pedicel. This is explained as the result of a displacement during growth by which part of the pedicel becomes united to the stem.

Occasionally, grooves or swellings are noticed at the base of the organ being abscissed where they correspond more or less exactly to the general position of the separation layer. Examples are given by Hannig (1913) for *Lycopersicum* and *Solanum tuberosum* and by Balls (1911) for *Gossypium*. Abscission may occasionally occur, according to Lloyd (1914*a*), above a small bract. According to these latter investigators, there is more often no external indication of the layer. Frequently, grooves bear no relation to the layer because in many cases of this kind (Hannig, 1913, for *Brunfelsia*) separation occurs a short distance distal to the groove.

From the above brief summary it is evident that in the case of axial abscission the separation layer is located at or near the base of an internode. Apparent exceptions are reported by Hannig (1913) in which it is seemingly located at the middle of an internode. It seems probable that a more critical re-examination might reveal the fact that even these exceptions accord with the general rule. In these cases, for example, the pedicel of the flowers in question might be composed of two internodes.

b. ORIGIN OF THE SEPARATION LAYER

Kubart (1906) states that the occurrence of the separation layer in all tyes of abscission may be explained in one of the three following ways: (*a*) the separation layer is preformed and represents simply a portion of the primary meristem which has remained in its original active state; (*b*) it represents a secondary meristem; (*c*) the primary meristem may function directly as a separation layer. The difference between *a* and *c* is only a difference in time, *c* being added to explain the origin of the separation layer in abscission of very young, embryonic tissues. In *a*, the separation layer is present at the base of the organ from the start of its development, but in *b* this layer has to be formed by a secondary meristem before abscission can occur. In *a*, cell divisions are not normally found preceding abscission, but in *b* and *c* they are. Mohl (1860), working on the fall of the flower in *Aesculus, Pavia, Lagenaria, Cucumis,* and *Ricinus,* states that the separation layer in these forms is of type *b*. Throughout his entire

work Mohl gives the general impression that it is necessary for a separation layer to be formed from a secondary meristem before abscission can occur. Wiesner (1871), working on leaf-fall in general, observes that the separation layer is not generally of type *b*, as Mohl believes, but more often of type *a*. According to Becquerel (1907), the separation layer is formed in the pedicel of *Nicotiana* from a secondary meristem (type *b*). In the cotton flower Balls (1911) finds that the separation layer is of type *b*, but according to Lloyd (1914*a* and 1916*b*) there is doubt as to this conclusion, since in the case of very young cotton flowers in which abscission occurs very suddenly, he finds only rarely that cell divisions do not precede abscission. Hannig (1913), for flower-fall in general, states that a separation layer of type *a* is always present but in certain species a secondary layer of type *b* may also be formed, through which separation may or may not occur. Hannig, differing from Becquerel (1907), points out that the separation layer in *Nicotiana* is of type *a*. Lloyd (1914*a*) and Loewi (1907) indicate that in general a layer of cells through which abscission is possible is more often of type *a* than of type *b*. They believe, however, that the separation layer is not a definite morphological structure but represents merely a physiological condition.

c. CYTOLOGY OF THE SEPARATION LAYER

Mohl (1860) describes the separation cells in the flower stalk as young, active, small cells which generally contain no starch. He also states that in most cases cell divisions are characteristic of the separation layer, i.e., that the separation layer is meristematic. Hoehnel (1880) finds that cell divisions are characteristic of the proximal portion of the separation zone in *Salix* and *Populus* but in the distal portion, where the separation layer is developed, these divisions are not so numerous. In some cases he finds separation taking place in the parenchyma, entirely outside the "zone" where there had been no cell divisions. The separation cells in *Nicotiana* are described by Becquerel (1907) as small, practically undifferentiated cells with large nuclei. In *Begonia, Fuschia, Mirabilis,* and *Impatiens* Hannig (1913) describes the tissue as secondary meristem (type *b*) with the cells rectangular in shape and arranged in more or less definite rows. In contrast to the above observations, he describes the cells as small, irregularly arranged and spherical in *Salvia, Solanum nigrum,* and *Nicotiana Tabacum*. In *Solanum nigrum* the separation layer consists

of two or three tiers of cells but in *N. Tabacum* the layer is made up of ten to fifteen tiers.

Hannig (1913), by means of various microchemical tests, can detect no chemical difference between the cell walls of the separation layer and those of the cells on either side. Lloyd (1914*a*), however, claims that the cell walls of the separation cells break down more quickly when treated with caustic potash than do the walls of normal cells. Starch grains are frequently noted by Hannig and Lloyd (1916*a*) as occurring in the separation cells, especially in the abscission of internodes by *Mirabilis Jalapa.*

An examination of the literature thus makes it evident that there has been a great difference noted in the various species in regard to the character of the separation cells. The one characteristic of these cells, however, to which there is no exception is that they are in an actively living condition.

2. The Process of Abscission

a. METHODS OF ABSCISSION

It has been found that in practically all cases of abscission the detaching of the organ is brought about by the separation of cells along the plane of the middle lamella. It is the method noted by Mohl (1860), Wiesner (1871), and Kubart (1906), who call it a process of maceration. Correns (1899) calls it a process of "schizolysis." Correns, however, in the same work describes a new and different method of abscission (rhexolysis) which he finds in mosses. In this latter method, separation is accomplished by a seemingly passive break of tissues irrespective of the position of cell walls. This may be the case in the style of cotton (cf. Lloyd, 1914*a*). This same method has been reported by Tison (1900) in the leaf of *Aristolochia Sipho,* although the evidence has been called in question by Lloyd and Loewi (1907). Still another type of abscission has been described by Hannig (1913) as a result of experiments on *Mirabilis* and *Oxybaphus.* In these plants he finds separation being brought about by a disorganization and dissolving away of a complete tissue. Lloyd (1916*a*), on the other hand, states that separation in these species is accomplished by cell separation and is thus true schizolysis. Hannig was doubtless confused in this case by the cell elongations which Lloyd observes and by which the membranes surrounding the protoplasts are drawn out exceedingly thin. Loewi (1907), working on

several genera, including *Cinnamomum* and *Euonymus,* notes and figures cell elongations similar to those figured by Lloyd (1916*a*). These cell elongations he finds so frequent and conspicuous that he proposes a distinct type of abscission, calling it a "Schlauchzell mechanismus."

Loewi, on the basis of his studies, seeks to classify the methods of cell separation in abscission under six different headings, which perhaps would be more appropriately presented under the next subject of consideration (the methods of cell separation); but since the author gave them as distinct methods of abscission they will be considered here. They are: (1) "round cell" mechanism; (2) dissolving of the middle lamella; (3) maceration; (4) turgescence; (5) cell elongations; (6) "hard cell" mechanism. They are to be considered merely as factors which, singly or in combinations, may enter in as a part of the normal process of cell separation. Loewi also claims that by controlling the temperature, humidity, and various other factors surrounding the plant he can influence it to such an extent as to change its method of cell separation.

b. METHOD OF CELL SEPARATION

It has been held by various investigators that the cell separation, almost universally connected with abscission, can be caused either by (*a*) chemical alteration and dissolving of the middle lamella or by (*b*) increase in cell turgor. This whole matter has received considerable attention, although very little direct evidence has been obtained. Wiesner (1871 and 1905) states that cell separation is caused by the dissolution of the middle lamella and by increased turgor. Kubart (1906) and Loewi (1907) agree entirely with Wiesner on this point. Strasburger (1913), Tison (1900), Lee (1911), Hannig (1913), and Lloyd (1916*a* and *b*) believe that cell separation is accomplished by the dissolution of the middle lamella. Practically all investigators have noticed the turgid appearance of the cells after separation, although this of course does not constitute evidence that the separation is due to increased turgor. Fitting (1911) claims that the separation is accomplished, at least in some cases, solely by an increased turgor of the separation cells. He bases his claim on the fact that abscission is very often too rapid to allow time for the dissolution of the middle lamella. He also mentions the fact that the separation cells are very often small, spherical cells, the type of cell which would respond most readily by an increase in cell turgor. On account of its

rapidity and regularity of reaction, Fitting claims that abscission is a semi-tropistic phenomenon and suggests the term *"Chorismus"* to designate this type of reaction.

It has been observed by Hannig and Fitting that the presence of various narcotic vapors in the atmosphere around certain species of plants causes their flowers or merely the petals to be thrown off. Various aspects of this general problem of the reaction of plant tissues to such agencies have been investigated. It has been determined by various plant physiologists that the presence of narcotic vapors, such as illuminating or acetylene gas, in the air around certain plant tissues causes the proportion of soluble carbohydrates within their cells to increase. This increase in the amount of soluble carbohydrates would indicate an increase in cell turgor. The question at once arises, whether or not this increase in turgor can effect complete separation or maceration of cells without the occurrence of chemical alteration in the walls. Richter (1908) resting his case on experimental evidence, throws some light on this problem. Various kinds of plant tissues which he subjected to acetylene vapors broke in pieces because of the maceration and collapse of the living cells within. He finds that in the case of the cells of tissues which are commonly rich in starch inclusions, such as the fruit of the snowberry and the potato tuber, the maceration is most complete. In the potato, for example, 3 to 5 mm. of material on the surface become completely macerated after being subjected to acetylene gas. According to Richter and Grafe (1911), the proportion of sugar in starchy seedlings subjected to acetylene gas is larger than in seedlings grown under normal conditions. In seedlings from oily seeds, however, the amount of sugar is decreased and the proportion of glycerine and fatty acids increased. The conclusion is therefore drawn that the subjection of plant tissues to narcotic vapors favors the hydrolysing process in the cells involved. The work of these two investigators goes to show that narcotic vapors may cause abscission by acting in either of the most important methods suggested as responsible for cell separation; they may increase cell turgor on the one hand or favor the hydrolysis of the middle lamella on the other.

Lloyd (1916*a*) presents evidence of chemical change in the cell walls of the separation layer before abscission. These cell walls stain in the usual manner with iodine, giving a light brownish color, but as abscission commences, they give a faint blue color when stained with iodine and washed out with water. Shortly before cell separa-

tion commences, Bismark brown and Ruthenium red fail to stain the primary and secondary cellulose membranes of the separation cells, although, when abscission does not occur, the entire cell wall is stained in the normal manner. The cells when separating seem, furthermore, to be surrounded only by the thin tertiary membranes. Lloyd, in his work, figures cells in the process of separation which show the dissolution of the primary and secondary membranes of the cell wall.

Various interpretations are given to the repeatedly observed occurrence of cell divisions preceding and accompanying abscission. Mohl (1860) expresses the opinion that cell divisions are generally necessary before abscission can occur. Investigators since his time have disproved the universal occurrence of cell divisions because they find more and more cases where no cell divisions occur. Lloyd (1914*a*) maintains that cell divisions are not of necessity correlated with abscission but are merely evidences of renewed growth and wound responses. As evidence he states that cell divisions are sometimes absent and sometimes present in the same species. He cites (1916*b*) the cotton plant as a typical example in which cell divisions are present in the abscission of older flowers in which the reaction to stimulus is slow. In young flowers and flower buds abscission may proceed without cell division. He further notes (1914*a*) that cell divisions sometimes precede and at other times follow abscission in a given species.

c. AGENCIES ACTIVE IN BRINGING ABOUT THE DISSOLUTION OF THE MIDDLE LAMELLA

Very few theories have been proposed to account for the dissolution of the middle lamella and practically no evidence of any kind has been submitted. Wiesner (1905) claims that in leaf-fall an organic acid, produced as a result of lessening of cell activity and stagnation of cell contents, acts on the middle lamella. His evidence for this statement has to do with obtaining acid reactions with litmus from cells at the base of the petiole during abscission. Kubart (1906) also obtains acid reactions at the base of the corolla in *Nicotiana* during abscission and, although agreeing with Wiesner that an organic acid probably causes the dissolution of the middle lamella, he also admits the possibility that an enzyme plays a part in the process. Lloyd (1916*b*) makes the statement that the dissolution of the middle lamella is a process of hydrolysis and although making no definite statement on the subject appears to take it for granted that an

enzyme of some kind is the active factor. Indeed, since all hydrolysing processes of living cells are now supposed to be due to the action of enzymes, there is no reason to suppose that the hydrolysis of the middle lamella does not conform to the general rule. For it is known that an enzyme, pectosinase, is capable of breaking down the pectose of which the middle lamella is composed. However, until more is known concerning the nature of this particular enzyme it remains impossible to get more definite evidence on this phase of the problem.

3. Abscission of the Corolla

Reiche (1885) gives an account of the fall of the corolla in a large number of species belonging to about forty-five families of the monocotyledons and dicotyledons. He finds that the corolla may be thrown off in one of three different ways: (1) by the activity of a small-celled separation layer; (2) through decay; (3) through increase in size of the ovary, thus tearing off the tissue involved at the base of the corolla. In many cases of true abscission—case 1 above—Reiche finds that the separation layer is preformed and ready to function at any moment. This represents a contradiction of Mohl's observations, according to which the fall of the corolla is usually due to the action of a separation layer formed shortly before fall. According to Reiche, the separation layer is very seldom morphologically differentiated from the neighboring tissue, but in a few cases he describes the separation layer as consisting of a layer of cells smaller than the neighboring cells on either side.

Kubart (1906), in his account of abscission of the corolla in several different species, describes and figures the process which takes place in *Nicotiana*. The separation layer in this genus he finds to be in no way morphologically differentiated, of indefinite shape, and located about 1 mm. above the base of the corolla tube. In this general region a large number of cells separate from one another, all the cells in cross-section taking part except the epidermal cells and the tracheae. Fitting (1911), in his work on the shedding of petals, describes the process of abscission in several genera, paying particular attention to *Erodium, Geranium, Linum, Helianthemum, Perlagonium*, and *Verbascum*. Separation in these cases takes place through a region of small, spherical cells rich in protoplasm. The separation layer is not sharply differentiated as compared with the tissues on either side but is located in a restricted region at the base of the petal.

He finds no cell divisions preceding or accompanying abscission. The process in premature abscission he finds differing in no way from that in normal abscission after fertilization. These conditions, he states, correspond more or less to those which he finds in the pedicel during flower-fall.

4. Time of Abscission

The time elapsing between anthesis and flower-fall in partially sterile F_1 species hybrids of *Nicotiana* and between emasculation at anthesis and fall in the case of their corresponding parents is discussed in a previous paper (Goodspeed and Kendall, 1916). It was there stated that the average time is about nineteen days in F_1 H154, seven in F_1 H179, five in *N. Tabacum* var. *macrophylla,* and thirteen in *N. sylvestris.* When we turn to the question of the reaction time in premature abscission occurring before the normal time as the result of sudden changes in external environmental conditions, we find that this subject has received only slight attention. According to Lloyd (1914*a*), the cotton "square" falls in one to twenty-two days after the weevil lays its eggs, the average time being eight days. In one experiment in which the ovary was cut transversely, Lloyd was able to cause one hundred per cent of the young bolls to fall in forty-eight hours and ninety per cent in twenty-four hours. Larger bolls take a longer time to respond to injury than do smaller ones, as a result of the development of the pedicel to a condition in which abscission meets greater resistance. Cotton "squares," he finds, take a longer time to respond than young bolls, the former shedding thirty-five to sixty per cent in thirty-six hours and the latter forty to seventy per cent in forty-eight hours. On the other hand, he obtains no evidence (1916*b*) that the reaction times are any shorter in small buds than in larger ones. The reaction times in cases where the injury is performed in the evening seem to be shorter by about twelve hours than in cases where the injury is performed in the morning. This difference he ascribes to the increase in turgidity which takes place during the night and which serves to hasten the reaction. Very severe injuries to the ovary, he finds, cause fall of young bolls quicker than less severe injuries. Injuries which are less severe than those mentioned above and performed so as to imitate the injury inflicted on the ovary by insect larvae caused shedding in three to six days, with most of the fall occurring on the fifth day. Summing up his entire results, Lloyd

(1916*b*) states that under field conditions the responses to all kinds of stimuli conducive to abscission become evident within ten days, with the maximum frequency below six days.

The actual time involved in the process of abscission (abscission time) has received even less attention than the problems discussed above. Fitting (1911) states that abscission time may occasionally be very short, forty-five seconds to five minutes in the petals of *Verbascum* and thirty seconds to six minutes in *Geranium*. Lloyd (1914*a* and 1916*b*) finds abscission after injury of the small cotton-boll taking place within four hours, the length of time depending somewhat on the age of the boll. In a previous paper (Goodspeed and Kendall, 1916) a general estimate of the abscission time was given and it was stated that normal abscission due to lack of fertilization takes place in *Nicotiana* hybrids in four to eight hours and premature abscission in one to four hours.

5. Experimental Induction of Abscission

According to Hannig and Loewi, abscission may be induced in two different ways. First by abnormal external conditions ("spontaneous" or premature abscission) and second by normal internal conditions at the normal time ("automatic" or normal abscission). We shall consider in the following summary of the literature only two aspects of induction of the first type.

a. Induction by narcotic vapors

Hannig (1913) reports a comparative study of the behavior of cut sprigs of different species of plants when subjected to laboratory air and to illuminating gas. He notes the fact that under either of the above conditions all the flowers and occasionally a few small shoots are abscissed. He finds, however, that not all the species in a given family behave similarly in response to these conditions. We are particularly interested in the Solanaceae and we may note that this family contained more species that detached their flowers in illuminating gas than any other of the families investigated by Hannig. According to Fitting (1911), narcotic vapors such as tobacco smoke, carbon dioxide, ether, chloroform or illuminating gas frequently cause premature abscission of the corolla. He notices, however, that ammonia or turpentine vapors fail to cause abscission. Brown and Escomb (1902) make the statement that *Nicotiana, Cucurbita,* and *Fuchsia* shed flowers and buds in air containing only 0.114 per cent carbon dioxide.

b. INDUCTION BY MECHANICAL INJURY

Becquerel (1907), in a brief paper on the effect of wounding flowers of *Nicotiana*, notes that even after fifteen days flowers without sepals, anthers, or stigmas do not fall. After the same length of time, flowers without corollas or flowers in which the corolla or stamens are only half removed, have fallen. He points out that this result is more conspicuous in young flowers but did not investigate this point sufficiently to arrive at any definite conclusions. According to Hannig, removal of various organs of flowers frequently causes abscission but wounding of the pedicel does not. He concludes, therefore, that injury itself does not cause abscission but only acts indirectly by interfering with important physiological processes in the treated tissues.

According to Lloyd (1914*a*), shedding of very young cotton-bolls can be induced by removal of the styles before pollination, but fall in this case can be assigned, as Fitting has shown, to lack of fertilization. It appears that in the cotton flower (Lloyd, 1916*b*) there is an inhibition period which starts with the opening of the corolla and during which premature abscission as the result of sudden stimuli very seldom occurs. Also, cotton-bolls larger than 30 mm. in diameter are very seldom shed under any conditions. Other results obtained by Lloyd on the effect of injury on the abscission of cotton flowers are discussed above under "Time of Abscission" (page 357). Lloyd (1914*b*) also notes the effect of injury on abscission of internodes in *Impatiens Sultani*. Plants of this species, when a cut is made across the stem, cast off the remainder of the severed internode. He gives results of experiments on the effect of different types of injury, noting that some severe injuries do not cause abscission. Gortner and Harris (1914) have obtained similar results with the same species. They find that when the cut is made across the internode, very close to the separation layer, abscission usually occurs, but occasionally it does not. They state, as does Lloyd, that the shape and location of the separation layer may vary slightly according to the type of injury.

c. THE DIRECT OR INDIRECT ACTION OF THE EXTERNAL STIMULUS

In all the above investigations the question naturally arises, whether the narcotic vapors and injuries or any stimulus conducive to abscission act indirectly through their influence on the physiological condition of the plant or directly, through their action on the cells of the separation zone. Most investigators, except Wiesner, ex-

press the opinion that atmospheric factors work directly in causing "spontaneous" abscission, although offering, so far as I can see, no evidence for this view. Fitting states that the external influence acts directly in most cases, but that the indirect action is apparent in forms which must build a separation layer before fall can occur. In regard to the action of injury, it seems to be the opinion of most investigators (Hannig, Bacquerel, Gortner and Harris) that the stimulus acts indirectly by interfering in some way with such important physiological processes as transpiration, respiration, or assimilation. On the other hand, if abscission is sometimes a semi-tropistic phenomenon, as Fitting has suggested, it is evident that injury may act directly in causing flower-fall.

TECHNIQUE

The results noted below were obtained largely from the examination of microscopic preparations made by the paraffin method, although this method was supplemented by free-hand sections mounted in water. In investigating the condition of the pedicel in some species (*Datura* sp., *Petunia* sp. and several species of *Nicotiana*) only free-hand sections were examined. For most microchemical studies fairly thick, free-hand sections are preferable. The material for sectioning in paraffin was killed and fixed in various concentrations of the chromo-acetic series and dehydration and infiltration were, in general, carried on very slowly. The free-hand sections were mounted in water without killing.

In cutting longitudinal sections of any kind all the pedicels were oriented so that the sections were cut parallel to the main stem of the inflorescence, in the plane formed by the pedicel and stem taken together. In studying the histology of the pedicel and the cytology of the separation layer and in studying the method of cell separation, these longitudinal sections were supplemented by cross sections in series through the base of the pedicel. It was impossible to cut very thin, longitudinal sections in paraffin without crushing or breaking the cells; most of these sections therefore were cut from 10μ to 15μ in thickness. For a similar reason, it was found necessary to cut thick sections (20μ to 25μ) of the pedicels of fruits in which mechanical tissue had developed. It was possible, however, to cut excellent paraffin sections from 5μ to 7μ in thickness in cross-section or longitudinally through the small cells of the separation zone. Since the cells of the

separation zone are very small, not much could be determined in regard to the dissolution of cell walls by means of thick, free-hand sections. The best results along this line were obtained from the thin paraffin sections of the separation zone, although in order to show the cell wall in its normal thickness it was necessary to use the free-hand sections. As a supplement to these sections, several points of interest were brought out by washing off the isolated cells from the end of freshly abscissed pedicels and mounting them for microscopic examination.

In most of the work the paraffin sections were stained in safranin and Delafield's haematoxylin. The free-hand sections were generally mounted in water and stained in iodine. In special instances other stains were used. Thus, in testing for chemical differences in the cell walls of the separation cells, several other stains, such as erythrosin, eosin, Bismark brown, gentian violet and Ruthenium red were used. It was found that for demonstrating the dissolution of cell walls aqueous methylene blue was an excellent stain to use. This stain was allowed to act overnight and the sections destained slightly in alcohol. Methylene blue was also an excellent stain for the isolated cells obtained as noted above. By fixing these cells to the slide with albumen fixative and staining with this stain, the thin membranous wall surrounding the protoplast can be distinctly seen.

Various methods, such as subjecting inflorescences to illuminating gas and mechanical injury, were used to bring about abscission. The best results were obtained in cases where abscission was induced by inserting shoots under a bell-jar containing from 1.5 per cent to 3 per cent illuminating gas. By using illuminating gas in this way and by taking sections of the pedicels at intervals it was possible to determine just when the first signs of abscission appeared in a certain percentage of gas. This time was definitely determined for certain species so that it was possible to get material killed and fixed at any desired stage in the process of abscission. It was found that the best results were obtained by killing and fixing the pedicels at about the time when abscission was known to be commencing.

HISTOLOGY AND CYTOLOGY OF THE PEDICEL

1. Histological and Cytological Conditions of the Mature Pedicel

a. NICOTIANA

The vascular system in *Nicotiana,* as in all the other genera examined, is characterized by intraxylary phloem. *Nicotiana* differs slightly from all others in that the xylem seems in cross-section to be composed of a continuous ring of radial strands of tracheæ rather than composed of a broken ring of distinct bundles. When a branch of the vascular system (fig. 1, *a*) containing twenty to thirty xylem strands is given off to the pedicel, it assumes the shape of a crescent in cross-section, with the opening of the crescent on the ventral side. A short distance distal to the groove which marks the separation zone (fig. 1, *b*), the crescent closes and throughout the remainder of the pedicel the vascular system forms a complete cylin-

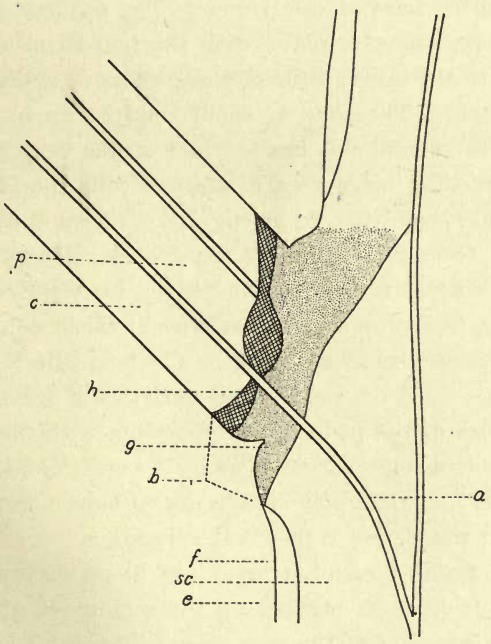

Fig. 1. Diagram of pedicel of *Nicotiana*

a—vascular system.
b—separation zone.
c—pedicel cortex.
sc—stem cortex.
e—epidermis.
f—chlorophyllous tissue.
g—groove.
h—separation layer.
p—pedicel pith.

der. The pith and cortex (fig. 1, *p* and *c*) are composed of large parenchyma cells which in the cortex are two or three times as long as wide, but in the pith are more nearly isodiametric. There is no mechanical tissue to be found in the floral pedicel but, as will be noted in more detail later, wood fibres are formed as soon as the fruit begins to develop. The epidermis of the pedicel (fig. 1, *e*) is typical but with a poorly developed cuticle, especially in the groove (fig. 1, *g*), where the cells are also much reduced longitudinally. Beneath the epidermis is a layer of small cells with very large intercellular spaces and an abundance of chloroplasts (fig. 1, *f*). This tissue stops a short distance proximal to the separation zone and does not continue in the pedicel. The layer of collenchyma which is commonly found in certain species just beneath this chlorophyl tissue is entirely absent in *Nicotiana,* or at least is very poorly developed.

Corresponding with the general region of the groove is an area of medullary and cortical cells which are smaller than corresponding cells on either the proximal or distal side of the groove. This region of small cells is homologous with the separation zone (fig. 1, *b*) and it extends across the base of the pedicel. The smallest cells are in the center of the region, in a plane with the bottom of the groove, and grade in size to the larger cells of the pith and cortex on either side (plate 49, fig. 1). The zone of small cells is ten to fifteen tiers of cells thick on the dorsal side but is wider on the ventral side, where it spreads out into the large area of storage cells found in the axil of the pedicel. The separation layer (fig. 1, *h*) is located five to seven tiers of cells distal from the bottom of the groove. Hanning reports this layer as occurring at the tip of the pedicel in *Nicotiana Langsdorffii,* but in all my experiments on two varieties of this species I find separation invariably occurring at the base of the pedicel in the position described above. All the species and varieties of *Nicotiana* examined show a structure of the pedicel corresponding with the above description except that in some varieties, as in those of *N. Bigelovii,* the separation zone is much thinner on the dorsal side. In such cases it is also noted that the groove is poorly developed.

The cells of the separation layer are in no way morphologically differentiated from those making up the remainder of the separation zone. Indeed, any cell of the zone seems capable of functioning as a separation cell. The separation cells are smaller than normal cortical cells and spherical in shape except in the vascular bundles, where they do not seem to be differentiated in size and are elongated parallel to the longitudinal axis of the pedicel. The cell walls are slightly thicker

than the walls of normal cortical cells, especially at the corners, thus giving the tissue a somewhat collenchymatous appearance. The smallest cells more proximal show this collenchymatous nature more strikingly than do the others. No difference in chemical composition could be detected, by means of microchemical tests using caustic potash, sulfuric acid, nitric acid, and various stains, between the cell walls of the separation cells and walls of other cortical cells. Other tests, however, indicated a difference in the nature of the cell contents in the two types of cells. Iodine frequently indicates the presence of starch in these cells and also colors the protoplasts a darker brown than in normal cells, showing that the separation cells are rich in protoplasm. The amount of starch in the cells, however, was found to be extremely variable, ranging from a total absence of starch to an abundance of it. Iodine green imparts to the protoplast of the separation cells a deep blue color in contrast with other cortical cells, which are not colored by this stain. The blue reaction is most prominent where the separation layer crosses the phloem. Other cells which react in the same way to this stain are the sieve tubes and companion cells and the storage cells in the axil of the pedicel.

b. LYCOPERSICUM

Conditions in *Lycopersicum* differ in certain respects from those existing in *Nicotiana*. In the former the separation zone (fig. 2, *a*) seems to be located at the middle of the pedicel and is marked externally by a swelling, as well as by the groove of the type already noted as characteristic of the pedicel of *Nicotiana*. This groove in the tomato is very deep (plate 53, fig. 1), reaching fully half the depth of the cortex, and is, furthermore, of about the same depth all the way round, differing in this respect from *Nicotiana*, where the groove is absent or poorly developed on the ventral side. The vascular system in *Lycopersicum* (fig. 2, *b*), in contrast with the condition in *Nicotiana*, is composed of scattered bundles of xylem which in this case do not form a crescent proximal to the groove but are in the form of a complete cylinder throughout the entire pedicel. Beneath the epidermis (fig.

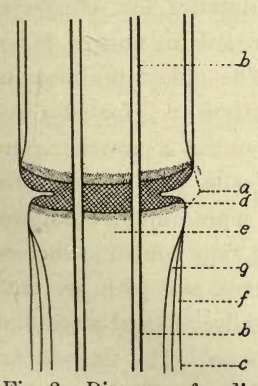

Fig. 2. Diagram of pedicel of *Lycopersicum*
a—separation zone.
b—vascular system.
c—epidermis.
d—separation layer.
e—pith.
f—chlorophyl-bearing tissue.
g—collenchyma

2, *c*) is the chlorophyl-bearing region of the cortex (fig. 2, *f*), such as occurs in *Nicotiana*, but in this case the tissue continues in the pedicel distal to the groove. Beneath this chlorophyl-bearing tissue is a layer of well-developed collenchyma (fig. 2, *g*) which however does not continue in the pedicel distal to the groove. The separation layer (fig. 2, *d*) consists of three to six tiers of cells and is located in a plane with the groove, differing in this respect from *Nicotiana*, where it is located a short distance distal to the groove. Corresponding to the condition in *Nicotiana*, the chief characteristic of the separation cells is their small size, spherical outline and active physiological condition.

c. OTHER GENERA OF THE *SOLANACEAE*

The condition of the pedicel, so far as the histology of the separation zone is concerned, was examined in several other species, a list of which is given below:

Solanum jasminioides
Solanum tuberosum
Solanum verbascifolium
Solanum umbelliferum
Solanum nigrum
Solanum marginatum
Cestrum fasciculatum
Iochroma tuberosa
Datura sanguineum
Salpichrora rhomboidea
Petunia hybrida
Salpiglossis sinuata
Lycium australis

The general condition of the pedicel of *Datura sanguineum* and *Petunia hybrida* is worth describing in some detail. The tissues of plants of *D. sanguineum* are more or less herbaceous in nature, large-celled and somewhat succulent throughout. The chlorophyl-bearing tissue which, in striking contrast with the condition in *Nicotiana* and *Lycopersicum* (figs. 1 and 2), is continuous over the separation zone, is composed of two rows of small, spherical cells just beneath the epidermis. Except for a layer of collenchyma, whose much elongated cells extend the entire length of the pedicel and thus continue the collenchyma through the separation layer, the cortex and pith are composed of more or less isodiametric, thin-walled cells. Floral abscission is as common in this species as it is in *Nicotiana*. The flowers are very large and furnish excellent material for a study of the cytology of abscission. Unfortunately not a sufficient number of flowers could be obtained to make possible any detailed study of this genus. It was noticed, however, that there is no region of small cells at the base of the pedicel within which separation occurs and that the separation cells are identical in size and shape with those on either side among

which separation does not occur. The separation layer here is located about 8 mm. distal to the base of the pedicel, with absolutely no external indication of its position. Microchemical tests, which in *Nicotiana* gave different reactions in the case of the separation zone and in the case of normal cortical cells, here fail to show any corresponding condition of differentiation.

Abscission has never been found to occur in *Petunia* or *Salpiglossis*, so that it is of interest to examine the histological condition of the base of the pedicel in these two species. They are practically identical with regard to the structure of the pedicel, so that the description given below can be taken as applying to both genera. The cortical cells of the pedicel pass into those of the stem without any groove or small-celled region. On the ventral side, however, is the region of small cells in the axis of the pedicel, which is more or less common to all flowers. The tissues of *Petunia* are not so soft and succulent as those of *Datura*, *Nicotiana*, and *Lycopersicum*. They tend rather to be dry and tough. The cells in the cortex and pith are also not so nearly isodiametric as in *Datura*, but are much elongated in a direction parallel with the long axis of the pedicel.

The condition in the other species mentioned above will be given only a general description. Abscission occurs in all the other species except *Salpichrora* and *Lycium* which, however, do not differ, in respect to the histology of the base of the pedicel, from any of the others. *Solanum tuberosum* resembles *Lycopersicum*. All the other species are similar in regard to the structure of the separation zone. There is in every case a general region of small cells extending across the base of the pedicel where the separation layer occurs.

3. Development of the Separation Zone in *Lycopersicum* and *Nicotiana*

a. LYCOPERSICUM

The development of the separation zone could be followed better in *Lycopersicum* than in *Nicotiana* because in the former the zone is not so close to the main axis of inflorescence. The problem here resolves itself into an effort to determine, by means of longitudinal sections of very young pedicels, how early in the development of the flower the groove and the differentiation in cell size of the separation cells appear. It was found that the development of the separation zone indicates the method by which the groove and differentiation in cell

size originate. The groove is fairly well developed (fig. 5) in young buds whose corolla is only 3 mm. in length, but is not so deep as in older buds. The cells of the separation zone at this stage are smaller than cells on either side, but the difference is not so prominent as in older flowers. In very small buds whose corolla is only 1 mm. in length or whose calyx is only 2 mm. long, the groove is just beginning to appear (fig. 4). In buds below this size (fig. 3) no groove or differentiation in cell size can be detected. Abscission can occur in these early stages, before the groove or differentiation in the size of the separation cells has appeared, as well as at any other stage. In these early stages the radial diameter of the cortex is much less, as compared with that of the pith, than in older flowers. It is evident, therefore, that the cells of the separation zone are small because they retain their original small size while the rest of the cortical cells increase in size. The fact that the groove is formed makes it probable that there have been few cell division, or none, in the separation zone of the cortex during the development of the bud. It was observed, however, that the cells of the separation zone in the pith retain their meristematic nature for a considerable period during the development

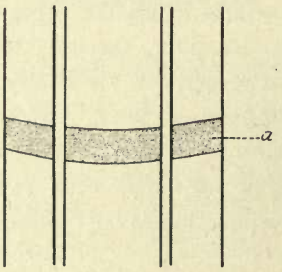

Fig. 3

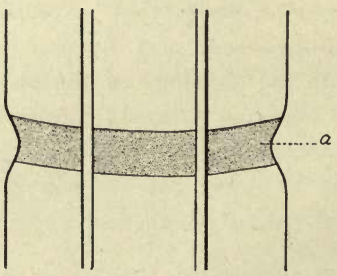

Fig. 4

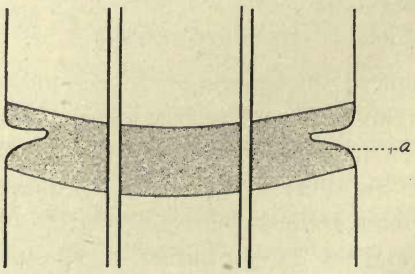

Fig. 5

a—separation zone

of the flower. They are at this time rectangular in shape, elongated perpendicularly to the long diameter of the pedicel, and arranged in longitudinal rows. In later stages, however, when the flower is at anthesis, or the fruit is forming, these cells have rounded up and become irregularly arranged, thus leaving rather large intercellular spaces.

b. NICOTIANA

The separation zone develops in *Nicotiana* much as it does in *Lycopersicum*. It was observed in very young buds—calyx 2 or 3 mm. or shorter—that no groove was present. In buds larger than these, the groove and small size of the separation cell is apparent, appearing first on the dorsal side of the pedicel. It is evident that in both these genera the groove and area of small cells are explained in the same way, i.e., by the fact that the normal cortical cells increase in size faster than do the cells of the separation zone. Since in both genera abscission can occur even before differentiation of any kind appears at the base of the pedicel, it is evident that the groove and small-celled region do not necessarily bear any relation to abscission. This statement is borne out by the fact that in *Datura* neither the groove nor the area of small cells is present and in *Nicotiana* separation occurs a short distance distal to the groove.

c. CONCLUSIONS FROM THE STUDY OF THE DEVELOPMENT OF THE SEPARATION ZONE

In view of the above discussion it is clear that the separation layer in *Lycopersicum, Nicotiana, Datura,* and probably in the other genera noted, originates according to the first method, *a,* proposed by Kubart (cf. page 350). That is to say, the separation layer represents merely a portion of the primary meristem which retains its original physiological capacities.

4. Increase in Size and Development of Mechanical Tissues in the Pedicel of *Nicotiana* and *Lycopersicum*

There is a marked increase in the size of the pedicel in both *Nicotiana* and *Lycopersicum* during the development of the fruit. It was found that during this development the diameter of the pith remains about the same, the actual increase in size being almost entirely confined to the cortex (cf. figs, 3, 4, and 5). This increase in the diameter of the cortex in the pedicel of *Nicotiana* is due, in the first place, to an increase in the size of the original cortical cells,

which in average cases measured about 20μ in diameter in the flower and about 40μ in the fruit. In the second place, it is due to four or five divisions of the cambium layer. This second factor in the increase in size of the pedicel becomes evident when a count is made of the cells between the phloem and tracheæ, the result giving approximately six cells in the flower and eleven in the fruit.

The increase in size of the pedicel of *Lycopersicum,* which is much more prominent than the increase in *Nicotiana,* can be explained in the same manner. In the former the increase in size, which in this case takes place almost entirely distal to the groove, may proceed to such an extent that the diameter of the pedicel of the fruit is two or three times that of the flower at anthesis. A measurement of the cortical cells in cross-section gave on the average 10μ in the flower and 28μ in the fruit. In this case only two or three divisions of the cambium occur; the cells resulting immediately show lignification.

The next subject of consideration is the development of mechanical tissue in the pedicel of *Nicotiana* and its relation to abscission. It will be remembered that there was no mechanical tissue noted in the pedicels of buds and flowers. Parallel with the development of the fruit, however, a continuous ring of mechanical tissue appears in the xylem of the pedicel. This mechanical tissue is evidently the result of a gradual lignification of the cells of the cambium and the outside portion of the xylem parenchyma. There is thus formed a continuous sheath of what may best be called wood-fibre tissue, in the form of a cylinder just outside the tracheal elements. These mechanical elements first appear in the tissues of the pedicel five or six days after anthesis, but since the lignification in these more distal tissues is merely the result of the spreading upwards of the lignification in the older parts of the plant, this period depends somewhat on the position of the flower on the inflorescence. It was noticed in *Nicotiana* that the wood-fibre tissue develops on both sides of the separation zone before appearing in the latter, but in time it becomes continuous through the separation layer. By a lignification of the cells between the two points of the crescent of wood in the separation zone, there is also a slight tendency to close this crescent on the ventral side.

Since abscission has not been observed to occur in lignified cells, the question at once arises whether the tough sheath of lignified cells which continues through the separation layer could hold the fruit on the stem even after actual abscission had occurred. Upon looking over any large number of plants in the field it will at once be evident

that such a condition of affairs very often exists. It will be found in many cases, especially on older plants, that although abscission has occurred in the cortex, as evidenced by the presence af a white, powdery substance at the base of the pedicel, the capsule is yet firm on the stem. Indeed, in certain hybrid tobaccos it is common to find most of the capsules in this abscissed condition. The fruit is supported in these cases by the tough mechanical elements of the wood, which also prevent the breaking of the tracheæ and protect the intraxylary phloem. In the pith the tissues may be in a somewhat abscissed condition, but since there is no way for these cells to escape through the sheath of wood they remain for some time in position before finally collapsing.

The development of mechanical tissues takes place in *Lycopersicum* in much the same manner as in *Nicotiana* but with the distinct difference that in the former the wood-fibre tissue does not become continuous through the separation layer. That is to say, in the tomato a break is left in the mechanical tissue in a plane with the bottom of the groove. It is evident here that abscission would cause fall of the fruit in any stage of its development, although in this case it happens that abscission very rarely occurs after two or three days past anthesis. A condition resembling this one in the tomato was observed in other berry-forming species of the Solanaceae such as *Cestrum fasciculatum* and *Solanum verbascifolium,* which often drop their immature fruits by abscission. Abscission, however, very seldom occurs in mature berries of these species, the fruit generally falling away from the receptacle above the calyx.

THE PROCESS OF ABSCISSION

1. General Description of the Process in Several Genera

a. NICOTIANA

The process of abscission in all the species of *Nicotiana* investigated conforms to the usual type involving separation and isolation of cells. Further details of the process were briefly discussed in a preliminary paper (Goodspeed and Kendall, 1916) for certain F_1 species hybrids of *Nicotiana.* It was there noted that cell separation starts in the dorsal side of the pedicel, in the cortex a short distance distal to the groove (pl. 49, fig. 1) and spreads from this point around to the ventral side. The first external indication seems to be a bulging of

the epidermis (pl. 49, fig. 2) over the tissue in which the process is taking place. Simultaneously with the start of abscission in the cortex, the process apparently originates independently in the pith (pl. 50, fig. 1). It was further noted that the number of cells concerned in the process, as a general rule, is greater in the hybrids than in their parents and also that this is true of "automatic" as compared with "spontaneous" abscission. Just beneath the epidermis the cells involved in separation were reported as being from five to ten tiers thick, but as the process approached the vascular tissue the separation layer was evidently reduced in thickness to not over one or two tiers of cells (pl. 52, fig. 1). In the pith a more or less spherical mass of cells is involved (pl. 50, fig. 1). When the separation is completed the flower may remain in position for some time, until the epidermis and tracheal elements are broken by some mechanical agency.

The exposed separation surface of the pedicel was stated to be convex in outline and slightly notched at the tip. Upon closer examination the surface itself was seen to be composed of the protruding, rounded ends of cells with here and there completely isolated cells and broken ends of spiral tracheæ. These isolated cells are apparently normal and do not markedly differ in form, size, or in the nature of their cell inclusions from the same cells before separation. The exposed surface of the attached portion of the pedicel is similar in appearance to that of the detached portion, but is more or less flat in outline. After separation the cells on this surface collapse and probably act as a protective layer.

Following the observations recorded above, which had to do largely with flower-fall in the F_1 species hybrids, a number of species have been investigated in an effort to determine whether or not their mode of abscission differs from that already described.

It may be noted at the start that no marked exceptions were found to the previously described condition, although at least two stages in the process of abscission have been found to be subject to considerable variation. The first of these stages has to do with the place of origin of the abscission process itself. An independent origin in the pith has been demonstrated to occur in a large number of species and occasionally it was found that the first evidences of abscission could be detected here before any similar evidences appeared in the cortex. Again, it was found in most species that cell separation starts first in the ventral cortex although other places of origin were found in several cases. Thus, in *Nicotiana Tabacum* "Maryland" and F_1 H36,

for example, the process originates on the ventral side and may even spread through the large area of storage cells in the axil of the pedicel before reaching the dorsal side. The distance distal from the bottom of the groove at which separation appears is also subject to variation. This variation, however, is not typical of certain species, since it may occur at different times in the same species, evidently as a result of an abnormal stimulation to abscission.

The second part of the process subject to variation has to do with the amount of tissue that may be concerned in actual cell separation. Abscission first becomes complete in a narrow plane between two or three tiers of cells across the pedicel and the flower can be easily shaken off at that time. If, however, the flower remains on the stem, and is kept turgid by the water rising in the unbroken tracheæ, cell separation spreads more and more widely through the tissues of the pedicel, especially in the pith and cortex. It is the extent to which this spreading normally proceeds that varies in the different species. When the process has spread to a considerable extent, a white ring formed by the isolated masses of cells can be seen with the naked eye at the base of the pedicel and a casual inspection indicates that the amount of this white substance varies in the different species. In most hybrids, except F_1 H179, there is more spreading in normal abscission than in pure species. In *Nicotiana quadrivalvis, N. Bigelovii,* and other similar species in which abscission very seldom occurs, no spreading takes place. Spreading, however, occurs to a remarkable extent in *N. Tabacum* "Maryland."

b. LYCOPERSICUM

We may say that, in general, abscission in *Lycopersicum* corresponds to that in *Nicotiana* and that the main points of distinction between the two arise only from the original differences in the separation zones (cf. page 364). In addition, attention must be called to the fact that quite frequently, in individual plants of the tomato, no true abscission occurs in normal flower-fall. In these cases the flower seems to be detached from the plant by a process which compares closely with that called exfoliation. There is no active cell separation and the flower simply wilts and dries back to the groove, where it hangs until broken off by some mechanical agency. The first indication of the process is the loss of chlorophyl in the pedicel, which gradually turns yellow, commencing at the tip and spreading proximal to the separation zone. It is possible that most of the flower-fall

noticed by agriculturists is of this type. Quite often, however, true abscission and this second type of flower-fall may both be found operative in the same plant or even in the same flower. "Spontaneous" flower-fall in the tomato is, of course, of the true abscission type.

Corresponding with the condition in *Nicotiana,* true abscission in *Lycopersicum* is seen to originate frequently in the pith. At any rate, the process goes on here independently of that in the cortex, since the final break is through the tracheæ and epidermis. Furthermore, separation takes place in a plane with the bottom of the groove (pl. 53, fig. 2) whereas, in *Nicotiana,* it takes place a short distance distal to the groove. Separation may at first take place between only two tiers of cells (pl. 53, fig. 2), but in time the process may spread until three or four tiers become involved in separation. However, there is no spreading of the process to a large number of cells, as is frequently seen in *Nicotiana,* so that one very seldom finds the white powdery substance at the point of separation. Also in contrast with the condition in *Nicotiana,* there is, as abscission progresses, no bulging of the epidermis which instead soon breaks in the bottom of the groove. Separation in the tomato takes place in such a way as to give the exposed separation surfaces the same general shape after abscission as in *Nicotiana,* that of the detached portion of the pedicel being convex and that of the remaining portion slightly concave.

c. DATURA

Conditions in *Datura* differ strikingly from those in the two species described above. This would be expected when one considers the great differences in the structure of the separation zones (cf. page 365). In *Datura* there is the usual chlorophyl-bearing tissue, which consists of two rows of small, perfectly isodiametric cells with large intercellular spaces, just beneath the epidermis. It will be remembered from the description on page 365 that this tissue in *Datura* continues the entire length of the pedicel and therefore, in contrast with the condition in *Nicotiana* and *Lycopersicum,* extends through the separation zone. The first sign of abscission is the maceration of this tissue as indicated by the appearance of a white color under the epidermis. The latter may as a result become detached from the tissues of the cortex for a distance of 2 cm. or more along the base of the pedicel. This is soon followed by a break over the separation layer and a curling back of the epidermis on either side, with most of the chlorophyl-bearing cortical tissues still attached to its inner surface.

After the break in the epidermis separation continues in the layer of collenchyma just beneath. The cells of the collenchyma layer, which are much elongated parallel to the long axis of the pedicel (five to eight times as long as wide), separate for a distance of about 0.3 mm. up and down the pedicel, involving only a few tiers of cells. It is evident that the cells of this tissue separate without difficulty, although not by any means as freely as the small spherical cells described above. The large, isodiametric, parenchyma cells of the cortex separate for a distance of 2 or 3 mm., involving many tiers of cells. The cells of the starch sheath, which are small and spherical, separate for a distance of 1 cm. or more, thus causing a longitudinal cavity to be formed just outside of the vascular bundles. In the latter, separation involves only two or three tiers of cells. Separation originates and continues in the pith independent of the process in the cortex, but involves about the same number of cells as in the parenchyma of the latter tissue. When separation has thus become complete, the weight of the flower is very often sufficient to break the tracheæ and cause the flower to fall to the ground.

Several important facts are brought out by this examination of abscission in *Datura*. In the first place, it shows that floral abscission can take place without any structure which might possibly be interpreted as a morphologically differentiated separation layer. In the second place, it indicates that cell separation is possible in several different types of living cells. It also shows that separation takes place more readily in small cells than in large ones and more readily in isodiametric cells than in elongated ones. The theory that the separation layer is not a morphologically differentiated structure, but represents a physiological condition (Lloyd and Loewi), could certainly be well applied in this case.

d. OTHER GENERA

The process of abscission in the other species listed on page 365 is essentially the same throughout. No indications were noted of cell divisions or elongations accompanying abscission. Separation is brought about by means of a separation of small and active cells located in the general region at the base of the pedicel. In all these forms the separation surface of the pedicel is convex in outline, so that the separation layer must lie in more or less of a crescent in the stem at the base of the pedicel. The main difference between these forms and the three that have been described in detail above is found

in the fact that in the former, with the exception of *Solanum tuberosum,* separation occurs in the stem at the very base of the pedicel, whereas in the latter three it occurs through the pedicel a varying distance from the base.

2. Method of Cell Separation

a. GENERAL REMARKS

It will be remembered that two theories have been proposed to account for the cell separation that is responsible for abscission. First, it is conceivable that cell separation may be caused by an increase in cell turgor, which causes the cells to round up and pull apart without any change taking place in the chemical nature of the middle lamella. Second, cell separation may be caused by a chemical dissolution of the middle lamella with or without an increase in cell turgor. The main difference between the two theories is that the second, in contrast with the first, maintains that chemical alteration of the middle lamella is always necessary before abscission can occur. The first theory gains support from the work of Fitting and the second from the work of Hannig, Lee, Strasburger, and Lloyd. Wiesner, Kubart, and Loewi believe that cell separation takes place by the action of both factors but that either factor may at times be the more important.

b. CYTOLOGICAL CHANGES ACCOMPANYING ABSCISSION

It was stated in a preliminary discussion (Goodspeed and Kendall, 1916) first, that no indication of cell divisions or elongations were observed accompanying abscission, and, second, that no evidence of the dissolution of the middle lamella had at that time been obtained. The first statement has been corroborated in that, during all the later experiments, no divisions or elongations have been observed in any of the described species. The dissolution of the primary cell membrane, however, because of more exact knowledge of the proper time to take sections and of more successful staining methods, has been fairly well established.

The main problem here was to determine by the use of various stains whether or not the primary and secondary cell membranes of the separation cells stain differently in the early stages of abscission than under normal conditions. This was a point which was found very difficult to determine, principally because of the fact that the

separation cells are, comparatively speaking, very small, but also because of the fact that the walls of these cells fail to show any stratification.

Iodine, Delafield's haematoxylin, Ruthenium red, Bismark brown, methylene blue, erythrosin, and eosin were used with little success in most cases. By using iodine, however, just as abscission is known to be commencing, a white streak may be seen across the section in the region of the separation layer. Upon careful examination it was decided that this white streak was due to the failure of most of the cell walls in the separation layer to take the stain. Although it is probable that with more careful examination the other stains mentioned above would give similar results, it was found that methylene blue was the only stain with which anything definite could be established. If a thin longitudinal section cut in paraffin as abscission is known to be starting, and stained in methylene blue, is examined (cf. page 361), it will be found that the walls of those cells in which separation is about to occur have remained almost entirely unstained. The protoplasts in these cases seem to be surrounded only by the thin tertiary membranes, between which is a streak of colorless material of varying width (pl. 51). Cell walls where separation is not expected to occur, however, stain a dark blue throughout in the normal manner.

An examination of freshly isolated cells washed off from the end of an abscissed pedicel shows that these cells are still turgid and active. It was impossible to determine whether these cells had increased in size, as compared with the size of similar cells before abscission, but it is evident that the increase, if any, had not been very great. The cells still contain their large nuclei, and occasional starch grains, and show after isolation no signs of degeneration even after several hours in water. In addition, these isolated cells appear to have retained their original shape. In the collenchyma of *Datura* the cells are from five to eight times as long as wide, and yet these cells retain their original shape when isolated, as a result of the dissolution of the middle lamellae. This isolation has evidently not been complete, since large masses of cells are seen still attached to each other. It is noticed that in all cases the protoplast is surrounded by an extremely thin membranous wall (pl. 52, fig. 3). It is also frequently noticed that the protoplast seems drawn away from the cell wall as if plasmolysis had occurred. It is possible that this appearance may be due simply to the gathering together of granules and the denser portion of the protoplasm in the center of the cell.

c. EXPERIMENTAL EVIDENCE FOR THE DISSOLUTION OF THE MIDDLE LAMELLA

It is supposed that the middle lamella, or primary cell membrane, is largely composed of calcium pectate, a calcium salt of pectic acid which has been given the general name pectose. The secondary cell membranes probably contain a larger proportion of cellulose with the pectose than is present in the primary membranes. This pectose, which is of course insoluble in water, is disorganized by a process of hydrolysis to form pectin. The pectin, which is a colorless mucilaginous substance, is readily soluble in water but is precipitated along with the proteids and enzymes of the protoplast by the addition of alcohol. Thus, if a water extract is made from separation zones during the first stages of abscission, one would expect to get a solution of several substances, among which would be the pectin produced by the dissolution of the pectose in the primary cell membranes. It might be expected that the amount of precipitate obtained from this extract with alcohol would be greater, provided the amount of other substances remained the same, than the amount of precipitate obtained in a similar manner from separation zones in which there had been no abscission and in which no pectin had been formed. Whether or not the increase in the amount of precipitate is due to the added pectin cannot of course be proven without actual chemical analysis, and such an analysis would be difficult because of the very small samples of material obtainable. However this may be, any difference in the amount of precipitate would be of interest.

This experiment and the two which follow are, as far as I have been able to determine, the first of their kind. Apart from this fact, their chief value probably lies in the fact that they suggest a line of investigation which, if carried on in more detail and with better facilities, will undoubtedly lead to important conclusions. These experiments were, however, carried on with as much care as possible and since the results of duplicate tests are in agreement, they give, as far as they go, dependable results.

After several experiments indicating the results given below, the following test experiment was performed:

Experiment 1.—Two water extracts of equal concentration were made from the lots of material. Lot A contained 200 small pieces of the pedicel in which the separation zone was located and in which abscission had started. Lot B contained an equal weight of a similar

number of pedicels in which no abscission had started. The extracts were made up to 10 cc. and the precipitate obtained with 60 cc. of 95 per cent alcohol. The precipitate weighed in the two lots:

A ..... 996 mg.
B ..... 903 mg.

One of the preliminary experiments performed with a weaker alcohol gave results which may or may not be of considerable importance. In this experiment a light, almost invisible precipitate formed in A and no precipitate in B. Whether or not the pectins precipitate in lower percentages of alcohol more readily than the other substances I have been unable to determine. At any rate, the precipitate in this case felt slimy and mucilaginous to the touch and might well have been the precipitated pectin approximately pure.

d. EVIDENCE FOR INCREASE IN TURGOR

It was stated along with other conclusions in the preliminary paper (Goodspeed and Kendall, 1916) that from the evidence at that time available it was probable that cell separation is caused merely by an increase in cell turgor, and throughout this later work it has been clear that increased turgor is present during abscission. In view of the evidence given above, however, it would seem that turgor can play only a secondary rôle, although the occurrence of increase in turgor must not be ignored.

The bulging of the epidermis frequently noted as accompanying abscission is evidence of increased internal pressure. In the pith the cells next to those which are separating are in a collapsed condition due to the pressure of the expanding separating cells. By various experiments it can be shown that humid conditions favor and severe drought prevents abscission. Richter and others have shown that narcotic vapors which cause abscission also cause increased turgor by increasing the proportion of sugar in starch-containing cells. This increase in cell turgor becomes so great as to cause complete maceration in certain types of tissues. The frequent presence of starch grains in the separation layer of *Nicotiana,* part of which are probably converted into sugar as a result of subjection to illuminating gas, indicates that there is probably an increase of turgor during abscission, at any rate when induced by illuminating gas.

On the other hand, a more extensive examination of abscission in certain plants indicates that all evidences of increased turgor may at

times be absent. Such cases might be explained by the absence of any considerable amount of starch in the cells concerned. Indeed, the starch grains usually noted in the separation layer can not at times be observed. This might also explain the fact that the bulging of the epidermis and collapse of cells in the pith usually accompanying abscission are sometimes absent. Also, starch grains are rarely observed in the separation cells of *Lycopersicum* and *Datura* and in these forms very little bulging of the epidermis occurs. Although humid conditions favor abscission and drought prevents the process, it has also been observed that drought has to be very severe before it produces such a result. Other evidences for increased turgor derived from the turgid appearance of the cells are mostly obtained after abscission has started and, granting that the cells are isolated by dissolution of the middle lamella, more or less expansion due to release of pressure is to be expected.

A critical examination of the separation cells during abscission brings out several facts, other than those mentioned in the above paragraph, which of themselves render inadmissible the theory that cell separation is brought about by increased turgor. These are as follows: 1. There seems to be no perceptible change in cell shape or size during separation. 2. The increase in size of the intercellular spaces does not necessarily take place first between the walls at the "corners" of the cells, but may appear first as a longitudinal streak between the lateral walls of the cells (pl. 51). 3. Cell isolation may be incomplete in large numbers of cells still remaining attached to each other. 4. Cell separation first becomes complete in a narrow plane between only two tiers of cells before spreading later to a larger number of cells. 5. The spreading of cell separation itself is obviously hard to explain on the basis of the turgor theory.

In view of the facts brought out in this discussion and the positive evidence for the dissolution of the primary membranes, it should be clear that increase in turgor, at least in the Solanaceae, is not the direct cause of cell separation. Undoubtedly there is often great increase in turgor during abscission, especially in certain types of cells, but this increase, instead of being the direct initiating factor, probably serves merely to hasten and facilitate the process.

e. EXPERIMENTS ON THE AMOUNT OF SUGAR IN THE STEM AND PEDICEL OF *NICOTIANA* DURING ABSCISSION

After several experiments, all of which indicated the results obtained below, the following experiment was performed. Experiment 2*a* was devised to show the change in the amount of sugar which occurs in the tissues of the pedicel during abscission. Experiment 2*b* was intended to show this same difference in a restricted region of the stem just proximal to the separation layer.

Experiment 2a. Lot A included 200 pedicels of flowers which had fallen a few minutes before being collected as a result of being subjected to illuminating gas. Lot B included 200 pedicels of flowers picked at the same time as those making up Lot A, but in which no abscission was induced. The water extracts made with 10 cc. from equal weights of the two lots were tested with surplus Fehlings solution. The precipitates formed upon boiling weighed:

A	68 mg.
B	95 mg.

Experiment 2b. This experiment was carried out in the same manner as experiment 2*a*, but the precipitates in this case were of such small quantity that no attempt was made to get actual figures as to their weights. It was clear, however, merely from an examination of the filter paper, that there was more precipitate in B than in A—just the reverse of Experiment 2*a*. The difference was evidently not as great as in the latter experiment.

Experiment 2*a* seems to indicate that during abscission there is a reduction of nearly one-third the normal amount of sugar in the pedicel. Other preliminary experiments performed as abscission was starting showed only a slight reduction in the amount of sugar in the pedicel. Thus possibly the withdrawal of sugar commences with the start of abscission. Experiment 2*b* indicates that there is probably a slight increase in the amount of sugar in the limited region proximal to the separation during abscission. It is possible that most of the withdrawn sugar is used as a source for the energy required in the active process of cell separation. The slight increase proximal to the separation layer also shows that there is probably an increase in cell turgor in the actual tissues which contain the separation layer, due to the conversion of starch into sugar.

f. POSSIBLE AGENCY ACTIVE IN THE DISSOLUTION OF THE MIDDLE LAMELLA

The pectose of the middle lamella may be broken down into the soluble pectin in three different ways—by the action of an acid, of an alkali, or of the enzyme pectosinase. Since it is doubtful whether alkaline reactions in living cells frequently get strong enough to affect the middle lamella, the probable active agency is limited to the acid or the enzyme action. Up to the last few years very little has been known about the action of enzymes concerned in pectic digestion. It has been natural, therefore, for investigators (cf. Wiesner, 1905, and Kubart, 1906) to consider the acid as probably the active agency. In this connection, it is well to state that I have obtained distinct acid reactions with litmus from the base of the corolla of *Nicotiana* during abscission. This would confirm Kubart, who, it will be remembered, obtained similar reactions from the corolla of *Nicotiana*. But in this case I sometimes obtained acid reactions from the corolla when in the normal condition. Since these observations offer no detailed evidence that acidity has increased during abscission to a degree higher than normal, their significance can well be doubted.

The tissues of *Datura* give a distinct acid reaction to litmus in the normal condition. Experiment 3 below shows a slight increase in acidity during abscission. No acid reactions of much intensity are given by the base of the pedicel of *Nicotiana* either in the normal or abscissed condition.

Experiment 3. Lot A contained the bases of three pedicels cut while abscission was going on. Lot B contained an equal weight (6 gm.) of the bases of three pedicels cut in the normal condition. These were extracted with water and the extracts made up to 10 cc. each. By titration with 10 per cent NaOH and phenolphtalein the following results were obtained:

A..... 0.75 cc. required to neutralize
B..... 0.6 cc. required to neutralize

A similar experiment on *Nicotiana* showed, however, that the normally low acidity of this genus is slightly reduced during abscission, as indicated by the following results:

A..... 0.25 cc. required to neutralize
B..... 0.37 cc. required to neutralize

The normal acidity in *Datura* is high, but it is doubtful whether the increase is large enough to account for the dissolution of the middle lamella. At any rate, it is certain that acidity does not enter into the problem in the pedicel of *Nicotiana*. We must, therefore, fall back upon the enzyme action as probably responsible for the process of cell separation.

Most hydrolysing processes characteristic of living cells are now supposed to be due to the action of enzymes of different kinds. It has been definitely claimed (cf. Atkins, 1916) that an enzyme which has been called pectosinase is capable of breaking down the pectose of which the middle lamella is composed. Add to this the fact that the action of enzymes has been shown, as has also the process of abscission, to be very sensitive to all kinds of changes in the external environment, and it is fairly safe to assume that the method of cell separation is fundamentally an enzyme problem. Irrefutable proof of this could be obtained only by testing for the activity of pectosinase during the early stages of abscission and by demonstrating the absence or inactivity of this enzyme in species where abscission does not occur.

ABSCISSION OF THE STYLE AND COROLLA

Abscission of the corolla in *Nicotiana* was described by Kubart and it may be said at once that the observations herein described agree entirely with his. Abscission of the corolla is brought about by the separation, without any previous cell divisions or elongations, of living cells at the base of the corolla tube. The separation layer, which is in no way morphologically differentiated from the neighboring tissue, is located about 1 mm. from the point of insertion of the corolla on the receptacle. It thus occurs in the distal part of a region where intergradation of cell shape, between the isodiametric cells of the receptacle and the more or less elongated cells of the corolla, is apparent. The separation cells which are in this region of intergradation are not isodiametric but are more or less elongated parallel to the long axis of the corolla. All the cells in cross-section of the

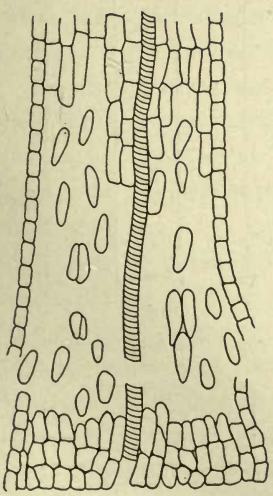

Fig. 6. Longitudinal radial section of the base of the corolla tube of *Nicotiana*, showing the method of abscission.

base of the corolla tube at about the level of the separation layer seem to be involved in the process except the epidermal cells and the tracheæ. The process of cell isolation in this case may spread up and down for quite a distance between the epidermis and tracheæ, thus involving a large number of cells (fig. 6).

Abscission of the corolla in *Datura* differs slightly from that in *Nicotiana*. As in the latter, there is no differentiated separation layer, separation occurring in cells which are not visibly different from other cells of the corolla. Cells more or less elongated are involved, as in *Nicotiana*, but in *Datura* the region of separating cells is limited to certain tissues—that is to say, not all the cells across the base of the corolla tube at about the level of the separation layer are involved in the process of abscission. The base of the corolla in *Datura* is characterized by distinct longitudinal ridges which alternate with deep grooves. Thus, a cross-section of a portion of the base of the corolla appears as in fig. 8. Cell separation fails to occur in the outside ridges at the level of the separation layer, so that, looking at the base of the corolla tube from the outside during abscission, one sees separate crescent-shaped regions of macerating cells alternating with cells which are not separating (fig. 7). This is explained when a cross-section is taken (fig. 8), which shows that several vascular bundles, the cells of which do not separate, are collected in the outside ridges.

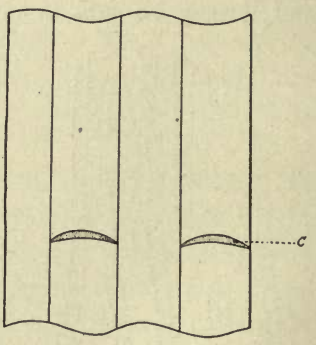

Fig. 7
c—region of separating cells.

Abscission of the style occurs normally in *Nicotiana* and *Datura* a short time before the corolla has fallen. So far as it was possible to determine, the process of abscission is exactly the same in the style as in the corolla. A separation of very small, more or less elongated cells takes place at the base of the style without any external indication such as frequently occurs in the pedicel of the

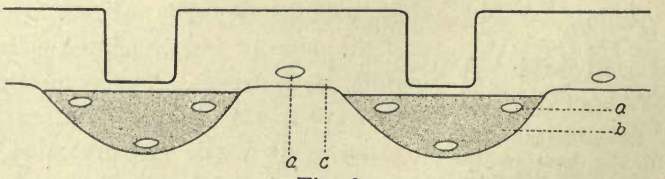

Fig. 8
a—vascular bundle.
c—region of cell separation.
b—region of no cell separation.

flower. As in the case of the corolla, there is no structure which might be interpreted as a differentiated separation layer. Separation occurs in a region of intergradation in cell shape between the spherical cells of the ovary and the cells of the style, which are elongated parallel with the long axis of the style.

TIME OF ABSCISSION

1. Reaction Time

a. REACTION TIME IN NORMAL ABSCISSION

The term "reaction time" is used in referring to two rather distinct subjects. First, we may have a reaction time represented by the time intervening between anthesis and normal abscission due to lack of fertilization. Second, we may have a reaction time which has to do with the period between the application of the stimulus and flower-fall in "spontaneous" abscission. The reaction times in normal abscission were discussed in an earlier communication in the case of two F_1 species hybrids of *Nicotiana* and their parents. The statements there made have been repeatedly verified and in addition a considerable amount of data has been accumulated in regard to the time of abscission in other species of *Nicotiana* and in the genus *Lycopersicum*. In the case of the former the observations were also made upon the abscission of the corolla, the effect of pollination on reaction time, and the reaction time in "spontaneous" abscission.

In determining the abscission times for the hybrid F_1 H154 (cf. page 386), a great variation was noted in the normal reaction time. In the case of the hybrid F_1 H179 (cf. page 386), however, and in other species or varieties investigated, very little variation in the time of abscission has been noted. The range of variation in these species and varieties practically always falls within two or three days and a large number of observations gives identical times, as far as the number of days is concerned, in the case of seven to ten flowers. There is a certain variation in the length of the reaction times in different flowers on the same plant, but the plants of a species do not differ from one another in their average reaction times. It was noticed that the figures were approximately the same whether the averages were based on the records of four or five flowers or of a considerably larger number; thus the results given in the following table may be considered conclusive. Where the number of flowers involved is less than four,

however, the results serve merely as approximate estimate of the abscission reaction time.

In obtaining the records tabulated below a separate tag was supplied for each flower. This tag was put on the flower at the beginning of the observation, the plant visited twice a day thereafter and records kept on the tags, which were left on the flower until the close of the observation. If a flower fell upon being tapped or shaken, abscission was considered to have occurred and the date was recorded on the tag, which was then collected. Similarly, in the case of the records for abscission of the corolla, a slight pull had to be applied before it could be determined whether abscission had occurred. As a means of preventing fertilization, the stigma was cut away in addi-

TABLE 1

Designation of species or variety	I Time from bud[1] to anthesis		II Time from pollination to mature fruit		III Time from pollination to abscission of corolla		IV Time from anthesis to abscission of corolla, unpollinated		V Time from anthesis to normal flower[3] fall	
	No. flowers	Avg. No. days	No. flowers	Avg. No. days	No. flowers	Avg. No. days	No. flowers	Avg. No. days	No. flowers	Avg. No. days
F_1 H38									10	18
F_1 H179	4	8	4	14	15	3	23	6	20	7
							8[2]	6		
F_1 H36					7	4	3	6		
N. sylvestris			10	11	6	4	9	6	9	15
							4[2]	6		
N. Tabacum "Maryland"			3	12	2	4	2	6	3	5
N. Bigelovii var. Wallacei			5	10	5	3	5	5	8	17
N. Bigelovii "Pomo"			5	19	5	2	6	5	6	no fall
N. Bigelovii var. typica									2	16
									3	no fall
N. Bigelovii (hybrid?)			4	21	1	4	2	8		
N. multivalvis			3	18	2	4			2	no fall
N. quadrivalvis			5	20	5	7	5	2	6	no fall
N. suaveolens					8	8	8	8	7	13
N. Sanderae			15	15	5	4	5	6	6	9
N. rustica var. brasilia							3	4	4	6
N. rustica (Winnebago)			7	6	7	3	4	4		
N. rustica var.?			4	15	4	2	5	3	6	5
Lycopersicum esculentum	4	6			4	3	4	8	17	9

[1] The buds recorded here were of such size that the corolla and calyx were of the same length.

[2] Sterile pollen applied to the stigma.

[3] By normal flower-fall is meant fall due to lack of fertilization.

tion to removing the anthers before anthesis. Various experiments had shown that such an operation on the flower does not induce abscission or affect its normal physiological condition to any great extent.

F_1 H154 is *Nicotiana Tabacum* var. *macrophylla* (U. C. B. G. 22/07) × *N. sylvestris* (U. C. B. G. 69/07). F_1 H179 is *N. Tabacum* "Cuba" (U. C. B. G. 200/14) × *N. sylvestris*. F_1 H36 is *N. sylvestris* × *N. Tabacum* var. *angustifolia* (U. C. G. B. 68/07).

The results given in table 1 indicate, in the first place, that the different species differ considerably in all the types of abscission reaction times considered, and, in the second place, that on the average, application of a fertile pollen to the stigma tends to shorten the time between anthesis and abscission of the corolla by two days. The one apparent exception to this statement is *Nicotiana suaveolens*, but in this case the pollinated flowers fell five or six days later than the corolla, indicating that growth of the pollen had not proceeded very far. Records on F_1 H179 and *N. sylvestris* indicate that sterile pollen does not have the same effect on abscission that fertile pollen does. This would seem to show that here the effect of pollination upon the postfloration phenomena is not due, as Fitting (1909) has found in orchids, to mere mechanical or chemical stimulation of the stigma by the pollen. This much being certain, the question still remains whether the results obtained depend upon fertilization or are due to the growth of the pollen tubes down through the style.

According to East (1915), working on self-sterility in *Nicotiana* hybrids, the pollen tubes reach the ovary, in cases of cross-pollination, three or four days after application of pollen to the stigma. Since in all cases recorded above cross-pollination was carried on and since in most cases the corolla was not thrown off until three or four days after application of pollen to the stigma, it is possible that fertilization is the important factor in shortening the time between anthesis and abscission of the corolla. In *N. quadrivalvis*, however, the corolla was thrown off within eighteen hours after pollination, whereas, when pollination is prevented, the corolla may remain on the flower for fifty-seven hours. If East's conclusions are correct, this would seem to indicate that the shortening of the reaction time in abscission of the corolla is due to some stimulation of the style by the pollen tubes and not to fertilization. This conclusion, however, could be doubted even here, because the style of *N. quadrivalvis* is very short, so that the pollen tubes might reach the ovary in a much shorter time than is

required in larger flowers. An attempt was made to get further data on this point by removing the style several hours after application of pollen before the pollen tubes could possibly have reached the ovary. This operation occasionally causes the whole flower to fall, and since in such cases abscission in the pedicel occurs before fall of the corolla, no results in regard to the latter organ are obtained. The possible effect of the operation on the abscission of the corolla was checked by control tests of unpollinated flowers in which the styles had also been removed. This can also be checked by a comparison with the periods of time given in table 1, column III.

It was found in three flowers of F_1 H179 that, when the style was removed three days after pollination, the corolla was, on the average, thrown off three days after anthesis. The control test for this experiment gave in three flowers an average of five days. Where the style was removed two days after anthesis, four flowers gave an average of three days. Where the style was removed one day after pollination, the corolla was abscissed in five flowers an average of three days after anthesis. A control test gave in this case an average of five days for five flowers. Finally, the style was removed in seven flowers seventeen hours after pollination. The seven flowers gave in this case an average of four days for the time between anthesis and fall of the corolla. A control test for this last case gave for five flowers an average of five days.

These experiments were repeated with *N. sylvestris*. In one case where the style was removed in three flowers two days after pollination, the corolla was thrown off on an average of four days after anthesis. A control test of this case gave an average of six days for three flowers. In another case the style was removed in three flowers one day after pollination. In this case the corolla was abscissed on an average of three days after anthesis.

The results given in the above paragraphs indicate definitely that it is the stimulation of stylar tissues caused by the growth of the pollen tubes which shortens the time between anthesis and abscission of the corolla. They also show that the removal of the style has no appreciable effect on the abscission of the corolla. It is evident from the results given that the influence of the pollen is seen as early as seventeen hours after pollination, and it is possible that the effect may be manifested even earlier. It is significant that the period given in the case where the style was removed seventeen hours after pollination is one day longer than in the case where the style was removed

twenty-four hours after pollination. This may possibly indicate that in the first case the influence of the pollen tubes has diminished, because of the shortening of the period which they have had for growth. If this is the case, it is reasonable to suppose that the influence of the growing pollen tube increases up to twenty-four hours after pollination as the pollen tube lengthens. Thus, at six hours after pollination it is possible that no effect of the pollen tubes would be noticeable, while twenty-four hours after pollination the entire influence of the growing pollen tube has been exerted.

The effect of pollination on the time between anthesis and flower-fall was tested by experiments similar to those described above. Results in such experiments are difficult to obtain because removal of the style frequently causes the premature fall of the flower. If the flower fell before abscission of the corolla, the fall was considered premature, as the result of the removal of the style, and the record of that particular flower not considered. Since under ordinary conditions pollinated flowers remain on the plant, it is to be expected that the stimulation of the stylar tissues by the pollen tubes, if it has any influence at all, would increase the length of time between anthesis and flower-fall. Granting the truth of this assumption, any reduction in time between anthesis and fall can be considered as the result of removal of the style.

In one test on ten flowers of F_1 H179, where the style was removed two days after pollination, flower-fall occurred on an average of seven days after anthesis. A control test in this case also gave seven days for ten flowers. This time is approximately the same (the actual average calculated to the tenth of a day was 6.7) as those given in table 1, column V, for the time between anthesis and normal flower-fall due to lack of fertilization. A similar test on six flowers of *N. sylvestris,* where the style was removed two days after pollination, gave an average of thirteen days. The time for this species in table 1, column V, is fifteen days.

These two records indicate that the stimulation of the stylar tissues by the growing pollen tubes has no effect on the time between anthesis and flower-fall. In the second case above, and also perhaps in the first, the stimulation of the style seems to have shortened the time somewhat, but in this case the result can be explained by the effect of the later removal of the style.

b. REACTION TIME IN "SPONTANEOUS" ABSCISSION

Exact data in regard to the reaction time can be given only in two definite cases. The observations in these cases were made on small shoots of the plant to be considered, which were placed in water and inserted under a bell-jar containing 1.5 per cent illuminating gas. After several hours, the material was shaken every fifteen minutes to determine when the first flower fell. F_1 H179 and *N. Tabacum* "Maryland" were selected as material for the experiments because these forms were found most sensitive and thus react regularly and quickly to stimuli. Abscission occurs in the pedicel of F_1 H179 seven hours after insertion into 1.5 per cent illuminating gas at a temperature of approximately 19° C. The smaller buds begin to fall first, but are followed in a short time by the open flowers. Abscission occurs in *N. Tabacum* "Maryland" in eight hours under the above conditions.

The remainder of the data having to do with the reaction time in spontaneous abscission is in the form of approximate estimates derived from the results of experiments on the induction of abscission. In the case of abscission induced by illuminating gas most species which shed their flowers in 1.5 per cent illuminating gas do so after ten or fifteen hours at room temperature.

There remains now to be considered the reaction time in cases of flower-fall due to mechanical injury. The results along this line are largely derived from tables 2, 3, 4, and 5, which, however, were arranged to show more particularly the comparative effect of different types of injury, as causing or not causing abscission in flowers of various ages. These tables might as well be presented under the heading "Experimental Induction of Abscission by Mechanical Injury" (page 405), but since it is necessary to draw certain conclusions from them in regard to the time of abscission they are presented and explained at this time.

Tables 2, 3, 4, and 5, which follow, serve to record the results of a number and variety of experiments all designed to show the relation of mechanical injury to abscission. It was very soon discovered while carrying on the experiments that the effect of injury depends to a large extent upon the age of the flower. Now the age of the flower can be most conveniently measured by determining the increase in size of growing parts such as the corolla and ovary. Thus it was necessary in each case to record the size of the flower—size being a

criterion of age—upon which the test was being made. This was done by noting on the tag which was supplied for each flower (cf. page 385) the length of the corolla in millimeters, the condition of the corolla, or any other condition of the flower which would serve to indicate its age. The period of development of the flower and fruit is divided into several arbitrary stages, each of which is designated by a Roman numeral in the second column of the tables. Where the number of flowers designated in the first column are nearly in the same stage of development only one numeral appears in the table, but where the range in size of the flowers is quite extensive two numerals appear, representing the range in size within which the flowers were found at the time of the experiment. The stages of floral development which each Roman numeral represents are given below.

Bud

I.....corolla 2 mm. to 5 mm. in length
II.....corolla 6 mm. to 10 mm. in length
III.....corolla 11 mm. to 15 mm. in length
IV.....corolla 16 mm. to 20 mm. in length
V.....corolla 21 mm. to 30 mm. in length
VI.....corolla 31 mm. to 40 mm. in length
VII.....corolla 41 mm. to 50 mm. in length

Flower

VIII.....corolla opening
IX.....anthesis
X.....2 days after anthesis
XI.....corolla withering

Fruit

Immature

XII.....fruit 5 mm. to 8 mm. in length
XIII.....fruit 9 mm. to 10 mm. in length

Mature

XIV.....fruit 11 mm. to 12 mm. in length

The operation of injuring the flower consisted largely in removing, by cutting away with a sharp safety razor blade, entire floral organs or parts of them. In some cases, however, organs were only slit longitudinally with a sharp knife or merely punctured with the point of a pair of forceps.

Several types of injury that remove the style, stigma or stamens before pollination may cause fall by preventing fertilization. It is evident, therefore, that fall occurring after such an operation performed on the flower before anthesis may be due to lack of fertilization and not to the injury. If, however, the fall occurs within the minimum time elapsing between anthesis and normal flower-fall due

to lack of fertilization, it can be safely concluded that the fall is due to the effect of the injury. This minimum time is about seven days for *N. Langsdorffii.* It can be safely said, therefore, that any fall occurring in less than seven days after injury to the flower near anthesis is due directly to the effect of the injury. In cases where the stamens or style are removed in flowers younger than those at anthesis,

TABLE 2

Effect of Different Types of Injury in Causing Flower fall in *N. Langsdorffii* var. *grandiflora*

	No. flowers	Size or condition of flowers	Injury to					Avg. No. days before fall of remaining organs
			Calyx	Corolla	Stamens	Pistil	Pedicel	
a	10	II-VII	all cut	all cut	all cut	all cut		1
	10	VII-XI	"	"	"	"		2
	10	XII-XIII	"	"	"	"		3
	2	XIV	"	"	"	"		no fall
b	4	I-II	½ cut	⅔ cut	"	style cut		7
	3	III-VII	"	"	"	"		7
	3	VIII-IX	"	"	"	"		7
	5	XI-XII	"	"	"	"		no fall
	2	XI-XII	"	"	"	style and part of ovary cut		5
	3	XIII-XIV	"	"	"	"		4
c	5	V-VII		½ cut	"	⅔ style cut		7
	5	VIII-IX		"	"	"		6
	2	XI		"	"	"		9
	3	XII		"	"	"		no fall
d	4	I	all cut					3
	1	II	"					no fall
	12	III-XII	"					"
e	2	V-VII	slit on sides to base	2 slit on 2 sides to base				7
	8	III-XI	"	"				no fall
	3	II	"	"		ovary slit		3
	3	V-VII	"	"		"		5
	3	IX	"	"		"		2
	2	XII	"	"		"		5
f	5	IX			anthers all cut			10
	2	V-VII			all cut			7
	3	VII-X			"			9
g	4	VIII				stigma cut		9
	2	V-VII				style cut		7
	2	VII-X				"		10
h	4	XIV	all cut			all cut		no fall
	2	XIII	½ cut			½ cut		2
	2	XIV	"			"		no fall
	4	XIII-XIV	slit to base			slit to base		"
i	10	II					slit to base	"
	3	IX					"	"
	6	I-VIII					1 cut ½ through	"
	8	II-XII					2 cuts ½ through	"

allowance must be made for the approximate number of days preceding anthesis. Thus, if a flower of the above species is injured three days before anthesis, the fall can not be assigned to the injury unless it occurs before ten days have elapsed. The minimum time for F_1 H179 is about five days; thus, any time of five days or more recorded on a flower, injured near anthesis, was considered as "no fall." The minimum time for *Lycopersicum* is about six days.

Finally, it is necessary to state that the process of reaction to the different types of injury recorded in the following tables was by no means impeded by low temperatures. *Nicotiana Langsdorffii* was tested out in a greenhouse where the average temperature approximated 75° F. The tests on F_1 H179 and *Lycopersicum* were performed in the botanical garden of the University during July and August, when the temperature was also comparatively high.

The following statement of results is derived in great part but not entirely from the foregoing tables. It has been noticed that cutting off the freshly opened flower at the tip of the pedicel causes the remainder of the pedicel to be thrown off in from ten to fifteen hours, but after the same operation on developed capsules the pedicel remains firm from thirty-six to ninety-six hours after the injury. Removal of the calyx causes the fall of buds in two or three days, depending upon the age of the bud. Removal of half the calyx together with two-thirds of the corolla and all the stamens causes fall in one to four days, depending upon the age of the flower. A

TABLE 3

EFFECT OF POLLINATION OF FLOWERS OF *N. Langsdorffii* var. *grandiflora* ON REACTION TO INJURY

	No. flowers	Pollination	Injury	Avg. No. days before fall
a	2	pollinated when injured	calyx and stamens cut	no fall
	2	not pollinated	"	10
b	4	pollinated when injured	calyx " ½ corolla cut	no fall
	5	not pollinated	"	8
		No. days after pollination when injured		
c	2	1	all organs cut at tip of pedicel	2
	2	2–6	"	2
	2	7–8	"	2
	3	2	½ calyx, ⅔ corolla, stamens, style cut	4
d	3	4–5	"	5
	2	6–7	"	2
	1	9	"	3
	1	9	"	no fall

transverse cut through the entire flower which passes through the middle of the ovary causes fall in one to two days. A similar operation in the case of maturing fruits changes the date of fall to four to eight days. Removal of half the corolla and all the stamens causes fall of buds in one day and the fall of young flowers in two to three days. Removal of the stamens or style in buds causes fall in

TABLE 4

Effect of Different Types of Injury in Causing Flower fall in F_1H179

	No. flowers	Size or condition of flowers	Injury to					Avg. No. days before fall of remaining organs
			Calyx	Corolla	Stamens	Pistil	Pedicel	
a	9	II-VIII	½ cut	⅔ cut	all cut	style cut		1
	6	XI-XV	"	"	"	"		no fall
b	4	III-VII		½ cut	"			1
	10	VIII-IX		"	"			no fall
c	10	V-VIII		"				"
d	9	I	all cut					2
	7	II	"					2
	3	II	"					no fall
	4	III-IV	"					3
	6	III-IV	"					no fall
	1	V	"					2
	4	V-VII	"					no fall
	2	IX	"					"
e	7	III-IV			"			2
	1	V			"			5
	3	V			"			no fall
	6	VI-VII			"			"
f	5	II-VIII				"		2
	4	VII-VIII				"		no fall
g	1	II	1 slit on 2 sides to base	1 slit on 2 sides to base				5
	1	II	"	"				no fall
	9	IV-VII	"	"				"
	9	II	2 slits on 2 sides to base	2 slits on 2 sides to base				1
	2	II-IV	"	"				4
	5	V-VII	"	"				no fall
h	5	II-V	punctured on both sides	punctured on both sides		ovary punctured, small hole		2
	3	VI-VII	"	"		"		no fall
	3	VII-XI	"	"		"		2
	3	II-III	"	"		"		2
	3	VI-X	"	"		"		2
i	15	III-XII					1 slit to base	no fall
	5						punctur'd many times	1
j	6	XIV	½ cut			capsule ½ cut		4
	3	XIV	"			"		no fall

two to four days. Severe injury of any kind to the ovary causes fall in one to two days.

The figures given above for the reaction time in cases of abscission following mechanical injury, together with a more detailed consideration of the tables, indicate that the reaction time, in general, does not depend so much on the type of injury as on the age of the flower concerned. What connection there is between the type of injury and the reaction time seems to be based, except in cases of injury to the ovary, on the relation of the amount of material removed to the amount remaining. Thus, cutting off the flower at the tip of the pedicel causes abscission of the remaining pedicel more quickly than any other type of injury. One exception to this statement is seen, as

TABLE 5

EFFECT OF DIFFERENT TYPES OF INJURY IN CAUSING FLOWER FALL IN *Lycopersicum esculentum*

	No. flowers	Size or condition of flowers	Injury to					Avg. No. days before fall of remaining organs
			Calyx	Corolla	Stamens	Pistil	Pedicel	
a	4	I	all cut					no fall
	4	II-VIII	"					"
	6	XII	"					"
b	3	XII				entire ovary cut		2
	3	XIII-XIV				ovary punctured 4 times on top		no fall
	1	XII				"		3
	4	XII				ovary punctured 4 times on side		2
	3	XIV				"		no fall
c	4	II	punctured at base	punctured		ovary punctured once on side		9
	4	VIII	"	"		"		4
d	4	II-VIII	½ cut	½ cut				no fall
	4	VIII-IX	"	"		"		5
	3	I-II		"		ovary ½ cut		1
	3	VIII	"	"		"		3
	2	IX	"	"		"		2
e	5	I-IX	"		all cut			no fall
f	5	VIII				style cut		5
	6	X-XI				"		no fall
g	5	VIII-XIV					slit	"
h	3	VIII		all cut	"			4
	5			"	"			no fall
	4	II-VIII		"				"

indicated above, in the case of injury to the ovary in which this organ may be merely punctured, without necessarily removing any material, yet abscission occurs in one to two days after the injury.

It has, on the other hand, been evident throughout all the abscission experiments that age of flower is the important factor in determining the reaction time, older flowers nearly always responding more slowly to stimulation by injury than younger ones. It will be seen, however, from the tables that there are occasionally individual exceptions to the general rule. These exceptions might be explained in a number of ways. For example, it is possible in the case of older flowers that the ovary, having increased in size, was accidentally cut in the operation of injury, thus adding the extra factor of stimulation of the ovary which in younger flowers would not be present. In general, such exceptions to the general rule indicate to what extent the normal or abnormal physiological conditions of the plant enter into the problem.

2. Abscission Time

The abscission time, or the actual time involved in the process of cell separation, was considered in a preliminary paper (Goodspeed and Kendall, 1916) wherein the minimum time in which abscission was known to have occurred was stated to be from four to eight hours in normal abscission and from one to four hours in "spontaneous" abscission. A few additional data are now at hand in the case of F_1 H179 and *Nicotiana Tabacum* "Maryland." These two forms, as has already been noted, are a little more sensitive than most *Nicotiana* varieties and normal abscission was found to take place in from three to six hours.

The time of cell separation in "spontaneous" abscission can be more exactly determined than that in normal abscission because of the regularity with which the plants respond to certain conditions of injury or to the presence of narcotic vapors. Data on this point were obtained in the following manner. Flowering shoots with flowers of different sizes were cut, placed in water and inserted under a bell-jar. Enough illuminating gas was then introduced under the jar to make 1.5 per cent approximately. The temperature during the experiment was practically constant at 19° C. After the shoot had been left in this abnormal atmosphere for five hours a few flowers were picked off at fifteen-minute intervals and free-hand sections made of their pedicels until flowers about the size of those which were being sec-

tioned began to fall. It was found that signs of abscission hardly ever appeared until thirty to forty minutes before actual fall occurred. This indicates that the actual process of cell separation in F_1 H179 takes place in from thirty to forty minutes. Experiments carried on in the same manner with *N. Tabacum* "Maryland" indicate that abscission here takes place in from forty-five to sixty minutes.

Both the reaction time of abscission and the actual abscission time are profoundly influenced by temperature and by humidity. Variation in the intensity of the illumination, however, seems to have no direct influence upon abscission. In comparing the effect of changes in temperature and humidity it was found that the results of experiments intended to show the time of abscission are far more dependent upon temperature than upon humidity. This is not because changes in humidity have little influence upon abscission but because such changes have to be very great indeed before bringing about any appreciable effect. Very slight changes in temperature, on the other hand, often influence abscission to a marked degree. Abscission goes on very actively under high temperatures and conversely very slowly under low temperatures. It starts in the case of F_1 H179 about seven hours after insertion in 1.5 per cent illuminating gas at a temperature of 19° C. If the same experiment be repeated in a temperature of approximately 9° C. abscission may not occur for fifteen to twenty-four hours.

Drought has to be quite severe before retarding abscission. There is no doubt, however, that wilted shoots will not drop flowers as quickly as fresh ones and if the wilting proceeds far enough no abscission will occur. This effect is all the more noticeable if the air around the wilted shoot is kept free from moisture.

EXPERIMENTAL INDUCTION OF ABSCISSION

1. Induction by Illuminating Gas

The first subject to be considered under this heading is the comparative effect of illuminating gas in causing abscission in several species of the Solanaceae. The method of determining this consisted largely in placing flowering shoots of the different species in water under bell-jars and introducing enough illuminating gas under the jars to make the percentage of narcotic vapors in the air around the plant 1.5. The temperature during the experiments was compara-

tively high, ranging from 15° to 20° C. The results, which were recorded approximately fifteen hours after subjection to the gas, are given in the following table:

TABLE 6

Species, variety, or hybrid	Amount of abscission, expressed almost entirely in terms of size of flowers thrown off
N. Tabacum var. macrophylla	all buds up to anthesis.
N. Tabacum "Maryland"	all flowers up to 4 or 5 days past anthesis.
F_1 H154	all buds up to opening of corolla.
F_1 H36	all buds and flowers.
F_1 H179	all buds and flowers.
N. glauca	young buds.
N. rustica var.?	buds up to anthesis.
N. rustica var.?	buds, flowers, and fruits.
N. Bigelovii var. Wallacei	no abscission.
N. Bigelovii "Pomo"	no abscission.
N. quadrivalvis	no abscission.
N. multivalvis	no abscission.
N. Sanderae	buds up to anthesis.
N. suaveolens	buds up to anthesis.
N. plumbaginifolia	buds up to opening of the corolla.
Solanum umbelliferum	small buds.
S. jasminioides	buds and flowers.
S. verbascifolium	no abscission.
S. nigrum	small buds.
Iochroma tuberosa	no abscission.
Cestrum fasciculatum	buds and flowers.
Lycopersicum esculentum var. pyriforme	no abscission.
L. esculentum var. vulgare	small buds and occasional flowers.
Petunia hybrida	no abscission.
Salpiglossis sinuata	no abscission.
Datura sanguineum	buds and flowers.
Salpichrora rhomboidea	no abscission.
Lycium australis	no abscission.

As might be expected, most of these varieties react to laboratory air in the same manner that they do to illuminating gas. In the case of laboratory air a longer time and higher temperature is generally required before the reaction occurs. All the species, with the exception of those which throw off only young buds, detach most of their flowers when left in laboratory air overnight. If a window or two is left open, allowing fresh aid to enter and at the same time lowering the temperature, no abscission occurs.

It was found that several of the species recorded above, in which no abscission or very little abscission occurred, detached more flowers when a larger percentage of gas was used or when subjected to 1.5 per cent gas for a longer time. Thus, both varieties of *Lycopersicum*

esculentum, Iochroma tuberosa, Solanum nigrum, and *S. verbascifolium,* upon subjection to 3 per cent illuminating gas for twenty hours, throw off all flowers up to those two or three days past anthesis. No abscission occurred, however, in any concentration of gas, in *Nicotiana Bigelovii, N. quadrivalvis, N. multivalvis, Lycium australis, Petunia hybrida, Salpiglossis stinuata,* or *Salpichrora rhomboidea.*

A peculiar condition exists in *Solanum umbelliferum,* which throws off buds in the illuminating gas but never under any conditions, including temperature or the presence of narcotic vapors, throws off flowers in which the corolla has fully opened. A corresponding condition seems to exist in *Nicotiana Tabacum* var. *macrophylla,* F_1 H154, *N. Sanderae, N. rustica* var. *brasilia,* and in one other variety of *N. rustica,* all of which seldom under any conditions detach fully opened flowers, although flowers up to that stage are freely abscissed. Thus there seems to be, in certain species and at about the time of the opening of the corolla, a sudden increase in resistance to the external stimulus which is causing abscission. In other species this sudden increase in resistance does not take place, abscission commonly occurring at any stage in the development of the flower or fruit and the increase in resistance taking place very gradually. In addition, there seems to be an intergradation of forms between those in which the increase in resistance takes place suddenly and those in which it takes place gradually.

The next subject to be taken up is a consideration of experiments 5, 6, 7, 8, and 9 on the induction of abscission in small isolated pieces of the pedicel. The main purpose of devising these experiments was to throw some light, if possible, on the direct or indirect action of the external factor in causing "spontaneous" abscission. The pedicel of F_1 H179 was again chosen as material for the following experiments,

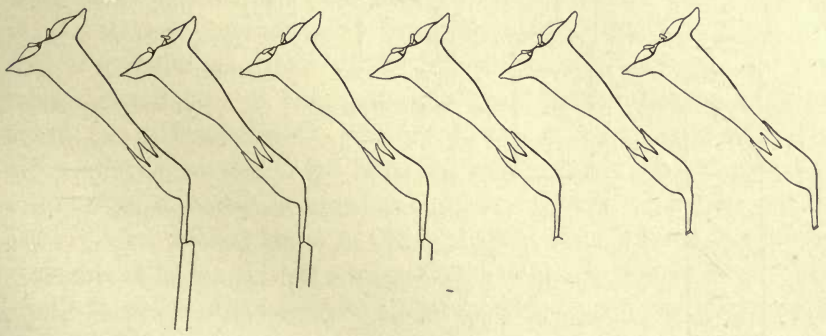

Fig. 9

largely because of the ease and regularity with which abscission is induced in this hybrid by sudden changes in the external environment.

Experiment 5.—This experiment was devised to discover the effect of reducing the volume of material proximal to the separation layer on the abscission of flowers of *Nicotiana* as induced by illuminating gas. Two series of flowers were cut as in figure 9. In the last two flowers represented on the right the cut was made less than 0.5 mm. from the separation layer. These flowers were then rolled in damp filter paper and left in 1.5 per cent illuminating gas overnight. After fifteen hours, abscission had occurred in all the flowers except the one represented on the extreme right in the figure. Abscission had occurred in one flower in which the cut had been made less than 0.5 mm. from the separation layer. The control to this experiment showed that abscission does not occur for several days in a series of flowers cut as in figure 9 and kept under normal conditions.

Experiment 6.—This experiment was devised to show the effect upon abscission of reducing the volume of material distal as well as proximal to the separation layer. In this case the flowers were cut off at varying distances from the separation layer, making the series shown in figure 10. The last two pieces on the right in this series were cut less than 0.5 mm. on each side of the separation layer so that the total length of the pieces was not much above 1 mm. In this experiment and in similar ones which follow it was necessary to keep

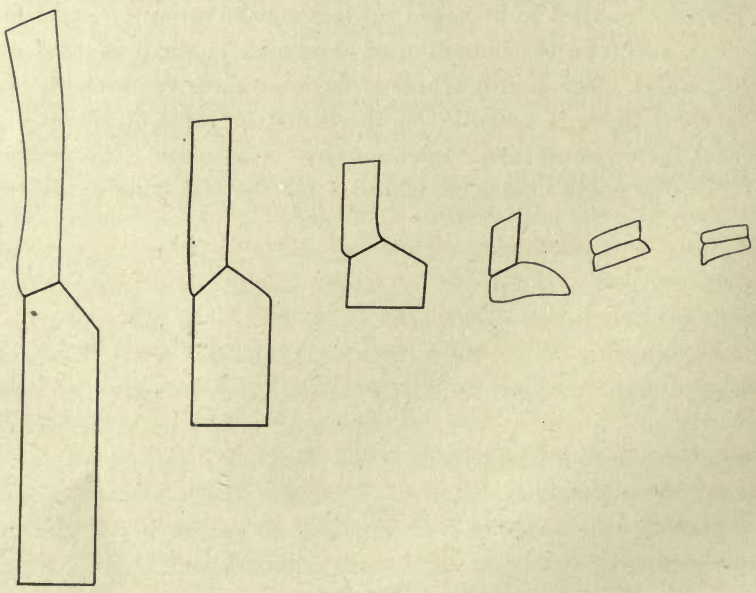

Fig. 10

the material moist. This was accomplished in various ways, but the best method was found to consist in placing the pieces on a long strip of filter paper one end of which rested in water. In this experiment abscission occurred after ten hours subjection to 1.5 per cent illuminating gas in all except the two pieces represented in the extreme right of figure 10. Abscission here took place in several pieces ranging from 1 mm. to 2 mm. in length. A microscopic examination of the separation surfaces indicated that the process of abscission corresponded entirely with normal abscission as it occurs in plants in the field. Experiments made in a similar manner upon *N. Tabacum* "Maryland" and *Lycopersicum* gave similar results. In the control, which consisted in keeping pieces of the pedicel as shown in figure 10 under normal atmospheric conditions, abscission occurred after about twenty hours, evidently as the result of no other stimulus than that caused through cutting off the flower by severing the pedicel. The reaction in the control, however, is much slower than in the case in which the added effect of the illuminating gas is operative, indicating that the latter factor, although it here serves merely to hasten the abscission process, has an effect of some kind on the tissues at the base of the pedicel.

Following these two experiments, a number of attempts were made in the same way to induce abscission in longitudinal free-hand sections of the pedicel cut for microscopical examination. It was soon discovered that the abscission process could be induced in the separation zone in thick longitudinal sections of the pedicel by subjecting them to high percentage (5 to 7 per cent) of illuminating gas. Cell separation in cross-sections through the separation zone could not be induced by any means at hand. The following experiments give more detailed results in this connection.

Experiment 7.—In this experiment, median, longitudinal sections of varying thickness were cut through the pedicels so that the plane of the sections corresponded with the plane formed by both the pedicel and the main axis of the inflorescence. These sections were subjected to 7 per cent illuminating gas, care being taken to keep them moist, but not submerged, throughout the entire experiment. The best arrangement was found to be one in which the sections rested in a thin film of water on one side but were exposed to the air on the other. After several hours in the 7 per cent illuminating gas, abscission started in the thicker sections but not in the thinner ones. The extent to which abscission proceeded depended upon the thickness of

the section. Abscission became complete in sections 0.3 mm. or more in thickness, the separation taking place in such a way that a slight bending or pulling motion sufficient to break the tracheæ divided the section into equal halves. In thinner sections, ranging from 0.3 mm. to 0.17 mm., abscission starts in the normal position but does not proceed to completion, the extent to which the process takes place depending, as has been said, upon the thickness of the section. In sections much below 0.17 mm. no signs of abscission appear. Also, if the thicker sections are shortened in length to any considerable extent by cutting off portions of the tissues from either side of the separation layer, abscission will not occur.

The process of abscission as it occurs in these sections corresponds exactly to the process in an entire pedicel. Cell separation starts independently in the pith and in the cortex, appearing first in that part of the cortex corresponding to the ventral region of the pedicel where, it will be remembered, abscission starts in the entire flower. When mounting the sections on an object slide for microscopical examination, the isolated cells in the pith lie in position but can be easily washed out with a small jet of water. In the cortex a break soon appears in the epidermis as the result of manipulation in mounting and a cavity is formed at that point as the result of the isolated cells of the cortex floating out in the water.

Experiment 7 was repeated in the case of *Datura* with similar results, except that in this case abscission was more active since it involved more cells, a situation which one might be led to expect because of the differences between the two species in the normal abscission of entire flowers. It will be remembered that the separation cells of the cortex in *Datura* are in no way distinguishable from other cortical cells; yet even in these sections separation occurs in a definitely predetermined position corresponding entirely with the position in abscission of the entire flower. It was even noticed that abscission started in these sections in the same tissues and in the same manner as in normal floral abscission.

After the thickness of the sections best adapted to obtaining results had been determined, the following experiment was performed on sections cut from different parts of the pedicel.

Experiment 8.—In this experiment a series of longitudinal sections of the pedicel were cut so that the plane of the sections was at right angles to that of the sections cut in Experiment 7. The first section was tangential, on the ventral side of the pedicel, and contained only the epidermis and a few tiers of cortical cells. Section 2 was also

tangential but contained a few tracheæ on one surface. Section 3 was more or less radial, containing two strands of vascular tissue on either side. Sections 4 and 5 were similar to sections 1 and 2. On subjecting these sections to illuminating gas it was noticed that abscission started first in sections 1, 2, and 3, appearing last in sections 4 and 5. This result is exactly parallel with the process as it occurs in normal abscission, where the process starts first in the ventral cortex and in the pith.

In passing, mention might be made of the peculiar reaction of the tangential sections 2 and 4, which were made up almost entirely of cortical cells with a few vascular elements on one side. When abscission occurred in these sections, a bending or bowing of the section was always noticed. This bending was always such that the tracheal tissue was on the concave side, as if the cells of the cortex had undergone considerable expansion while the cells of the vascular tissue retained their original size. From the work of Richter and others, it may be expected that subjection of portions of plant tissues to illuminating gas would cause an increase in turgor in the cells concerned. Thus, it is probable that the bending of the sections, as described above, is due to the increase in turgor of the cortical cells caused by the narcotic effect of the illuminating gas. The extent of the bending was such that most of the cells in the cortex as well as the separation cells must have been involved in the process. On repeating the above experiment with *Datura*, a similar bending of the tangential sections was even more pronounced than in *Nicotiana*.

Experiment 9.—As mentioned above, efforts to induce abscission failed in thin sections. The sections in Experiment 9 were cut so that they were thin in the separation layer but thick on either side. Both surfaces of these sections were thus cut slightly concave so that the sections were thickest at the ends and thinnest in the middle, where the separation zone was located. The sections were then subjected to 7 per cent illuminating gas as in Experiment 7. It was not possible to cut very thin free-hand sections of the shape described, but it was demonstrated without a doubt that abscission occurred in sections of this peculiar shape which were thinner in the separation zone than those in Experiment 7 where abscission had failed to occur.

Certain conclusions which can be drawn from experiments 5, 6, 7, 8, and 9 are given below.

1. Abscission can be induced by allowing the external factor to act directly upon the cells in the vicinity of the separation zone (Expts. 6, 7, and 8).

2. Abscission induced by the above methods in isolated pieces must be independent of transportation of material from the rest of the plant.

3. The fact that abscission cannot be induced in thick cross-sections of the separation zone shows that cell separation cannot be induced by the action of the external factor directly on the separation cells.

4. It is necessary that a certain proportion of the tissues of the pedicel be in intercellular connection with the cells of the separation zone before cell separation will occur, but this proportion is surprisingly small (Expts. 7, 8, and 9).

5. There is evidently increase in turgor in all the cortical cells of the pedicel during abscission induced by the above method (Expt. 8).

2. Action of Acids on the Separation Cells of *Nicotiana*

Under this heading a description will be given of the effect of mineral acids on small isolated pieces such as were used in experiments 6, 7, 8, and 9. It was stated above (page 364) that by the use of two mineral acids together with several stains, no chemical difference could be detected between the cell walls of the separation cells and those of normal cortical cells. The present work represents an attempt to determine, by experimental means and by watching through the microscope the action of acids on cell walls, whether the cell membranes of the separation cells are more subject to hydrolysis than those of normal cortical cells.

Experiment 10.—Small pieces of the pedicel were prepared as in figure 10. These pieces were boiled for one or two minutes in 4 per cent hydrochloric acid and then washed in water. Upon examination it was found that the pieces could be separated into halves through the separation zone by a slight pulling or bending motion. Microscopic examination of the separation surfaces showed that the break through the cells of the separation zone had taken place along the plane of the middle lamellae of their walls. This same type of separation was brought about without boiling when 10 per cent nitric or hydrochloric acid was allowed to act on the pedicels for approximately five minutes. When longitudinal sections are used in place of entire pedicles, the same results are obtained but much more rapidly. It was also noticed that separation under these latter conditions takes place more quickly in younger pedicels than in older ones. In the pedicels of fully developed fruits no separation could be induced, but in those of

immature fruits separation occurred in the cortex but failed to take place within the vascular cylinder.

Experiment 10 at first glance would seem to indicate that the cell walls of the separation cells are more subject to hydrolysis than normal cortical cells. Another interpretation is possible, however. Actual separation which takes place through the separation zone may be due to the fact that the cells in this zone are small and have a tendency to be isodiametric, whereas the remaining cells of the cortex are larger and are elongated parallel to the long axis of the pedicel. Hydrolysis of the cell walls may go on with equal rapidity in all the cortical cells at the base of the pedicel, yet upon bending or pulling separation may take place through the region of isodiametric cells because of the interlocking of the elongated cells in the rest of the cortex. An attempt was made to gain further evidence on this point by observing through the microscope the action of acids on the cell walls of the tissues concerned. When the action of the acids is thus observed, the walls are seen to soften and to swell to two or three times their normal thickness. This effect is all the more noticeable if the walls initially are comparatively thick. Now, since the cells of the separation zone are small and somewhat collenchymatous, or at least have thicker walls than normal cortical cells, the process of swelling in the cell wall is most conspicuous in that region. Indeed, hardly any swelling can be perceived as a result of the acid treatment in the cell walls of normal parenchyma cells of the cortex. However, when a form such as *Lycopersicum* is examined in which there is a distinct layer of collenchyma beneath the epidermis for the entire length of the pedicel, this collenchyma appears to be affected at the same time and in the same manner as the cells of the separation zone of *Nicotiana*. Also in *Nicotiana* there seems to be a certain amount of similarity in reaction to acids between the smaller cells of the cortex just beneath the epidermis and those of the separation zone. The conclusion can thus be drawn that the cell walls of the separation cells are no more readily hydrolyzed than those of normal collenchymatous tissues. Of course, the fact still remains that the collenchyma of the cortex may be more subject to hydrolysis than the cortical parenchyma. Now the small cells of the separation zone not only extend across the base of the pedicel but also spread throughout the general region at the base of that organ; it was therefore noticed that the swelling of cell walls was by no means confined to cells of the separation layer but was more or less prominent throughout the whole general region at the base of the pedicel.

The general results of these observations are in a sense negative and seem to indicate that the walls of the separation cells are no more subject to hydrolysis than the walls on either side. This, of course, does not preclude the possibility that a difference exists which is too slight to be detected. It appears, however, that the general region at the base of the pedicel may be more subject to hydrolysis than the more distant portions.

3. Induction by Mechanical Injury

The results of experiments on the induction of abscission by mechanical injury are recorded in tables 2, 3, 4, and 5, which have already been considered under the heading, "Time of Abscission" (page 384).

Several facts of interest brought out by table 2, which deals with *Nicotiana Langsdorffii* var. *grandiflora,* are summarized below.

1. It appears that removal of or injury to the capsule does not cause abscission in mature fruits (table 2, *a, b,* and *h;* table 3, *c* and *d*). The same types of injury generally do cause abscission in immature fruits.

2. It seems that a transverse cut completely through the flower at the distal end of the calyx causes abscission only in buds or flowers near anthesis (table 2, *c*). It appears, however, that such a cut proximal to the distal end of the calyx causes abscission in flowers several days past anthesis as well as in buds (table 2, *a, b*).

3. Removal of the entire calyx causes fall in very young buds only (table 2, *d*).

4. It seems that slitting both the corolla and calyx longitudinally on both sides from tip to base does not induce abscission even in young buds (table 2, *e*).

5. Entire removal of the style or stamens causes fall only in young buds (table 2, *f* and *g*).

6. It appears that injuries to the pedicel do not cause abscission, provided the flower is not entirely cut away (table 2, *i*). Just here it is worth mentioning that two of the pedicels cut transversely as recorded in table 2, *i,* were cut so deep that the flowers bent over and hung only by a few vascular strands and cortical cells. The wound healed over, however, and the two flowers matured with the rest.

7. It is evident that injuries which reach the ovary are much more effective in causing abscission than injuries affecting the other parts of the flower (table 2, *b* and *e*).

8. Fertilization has no influence whatever in preventing abscission when the latter is induced by a transverse cut completely through the flower at the base or middle of the calyx (table 3, *c* and *d*).

9. Certain types of injury, such as entire removal of the calyx and stamens or removal of the entire calyx and half the corolla, evidently cause abscission only by preventing fertilization (table 3, *a* and *b*).

Taking up now the results given in table 4, which dealt with F_1 H179, it will be seen that this hybrid is more sensitive to injury than is *N. Langsdorffii*. Nevertheless, it is very plain that the general conclusions announced above for this latter species hold for F_1 H179 also. There follows a partial summary of the results in table 4 and a comparison of these results with those obtained in the experiments on *N. Langsdorffii*.

1. It seems that removal of the calyx causes fall of much larger buds than in *N. Langsdorffii* (table 4, *d*).

2. F_1 H179 is evidently much more sensitive in its abscission reaction to a transverse cut through the flower at the middle of the calyx than *N. Langsdorffii* (table 4, *a*).

3. It would seem that slitting the calyx and corolla even to the extent of dividing these organs into four longitudinal strips does not, as a general rule, cause abscission. Such an injury does cause abscission only in extremely small buds (table 4, *g*).

4. It appears that puncturing the calyx, corolla and ovary so that a hole is formed about 2 mm. in diameter in the latter organ causes fall in flowers of all sizes up to two or three days past anthesis (table 4, *h*). Since it is evident that such a hole through the calyx and corolla alone would not cause abscission (table 4, *g*), abscission in this case must be induced by injury to the ovary.

5. It is evident that a slit completely through the pedicel for its entire length fails to cause fall in buds or open flowers, but where an effort is made to destroy completely the connection between the flower and stem abscission will occur (table 4, *i*).

6. Removal of the style or stamens, as a general rule, causes fall only in young buds, but removal of the former organ is probably more effective in causing flower-fall than removal of the stamens (table 4, *e* and *f*). On the other hand, where half the corolla is removed along with the stamens fall occurs in larger buds than where only the latter organs are removed (table 4, *b*).

7. Removal of only half the corolla apparently does not induce abscission (table 4, *c*).

8. Mature capsules of F_1 H179 are apparently more sensitive to injury than those of *N. Langsdorffii* (table 4, *j*).

The table dealing with the experiments on *Lycopersicum* indicates that flowers of this genus are remarkably resistant to injury, fall occurring only as the result of stimulation when the ovary is injured (table 5, *c* and *d*). Since a large number of tomato flowers are normally abscissed from the different inflorescences on a plant, the several exceptions to the above statement noted in the table probably demonstrate to what extent the normal physiological condition of the plant affects the matter. It seems to be the opinion of most gardeners who are familiar with the tomato plant that floral abscission in this species is more dependent upon soil conditions than upon injury or sudden changes in climatic conditions. It would seem, however, that injuries to very young fruits normally cause fall, but in this case a stage of development is soon reached at which injury to the berry has no effect in inducing abscission (table 5, *f*).

Taking the general results of all the experiments into consideration, it is seen, in the first place, that where injury of a certain type causes fall, a stage of development of the flower is soon reached beyond which the injury no longer causes fall. The increase in resistance to the stimulus of mechanical injury takes place gradually in the species investigated, but some of the species are much more resistant than others. In the second place, injuries to the ovary generally cause flower-fall. Thirdly, whether or not flower-fall occurs as a result of injury to other flower parts depends in some way upon the quantity of material removed. Fourthly, injury to the pedicel does not cause abscission unless it breaks entirely the cellular connection between flower and stem. Lastly, it is improbable that fall induced by injury is due to checking the transpiration stream, since injury to the ovary could have no such effect. Also, a cut across the pedicel so that the flower hangs by only a few tracheæ must check transpiration from the flower considerably, yet in this case no abscission occurs.

It was suggested by Bequerel that injury might cause abscission by checking the transpiration stream which passes up through the pedicel. Considerable doubt has already been cast on this point in the above discussion. In order to throw more light on this question the following experiment was performed in an effort to determine whether checking the transpiration stream of itself and unaccompanied by mechanical injury would cause abscission.

Experiment 12.—As a means of checking transpiration from the flower a coating of paraffin seemed desirable because it hardens

quickly, thus permitting several coats to be applied. It was doubtful whether other substances, such as lard, cocoa butter or vaseline, which might have been used, would not have been prevented from completely covering the flower in one coating by the presence of numerous hairs and glandular fluid on the calyx. In this experiment flowers were immersed in melted paraffin to within a millimeter of the separation zone and allowed to stand in water under normal atmospheric conditions. As a test for abscission, the shoot was shaken or individual flowers tapped from time to time. It was found that several *Nicotiana* varieties and hybrids differed in their reaction to this treatment as they did in their reaction to illuminating gas. In *N. Tabacum* "Maryland," for example, paraffining the flowers failed to cause abscission for six days, at the end of which time the flowers began to fall, as did those of the control. Some varieties, however, under such treatment, throw off buds at the end of twenty-four hours, but open flowers of the same varieties are never shed. Whether or not the buds fell in these varieties depended largely on the temperature, at lower temperatures no fall occurring. Also, in cases where abscission of buds did occur it was evident that something was actually impeding the process; none of the white substance formed by the isolated cells was seen at the base of the pedicel and the buds had to be shaken or tapped quite severely before they fell.

The results of Experiment 12 and the various observations on the induction of abscission by mechanical injury render it extremely unlikely that checking the transpiration stream is ever a direct cause of abscission. The few cases recorded above in which such a condition seems to cause abscission can be better explained by the action of some other factor than that of interference with transpiration.

In connection with these experiments upon the effect of checking transpiration the results of Lloyd and Balls on the effect of root pruning, etc., in cotton must be mentioned. It was found that a premature shedding of flowers and young bolls followed root pruning and further that, in general, there is a relation between boll-shedding and the rise and fall of the water-table. Proof positive is not supplied that root pruning causes fall of flowers by reducing the water supply of the plant body, and any number of other factors may enter in after such mutilation to bring about, in part at least, such a result. Experiments reported in the present paper seem to leave no doubt that, in *Nicotiana* at least, temperature is a more important factor in controlling abscission than water supply.

4. The Ability of Certain Species to Throw off Pedicels from which All the Floral Organs Have Been Removed, as Related to the Induction of Abscission by Mechanical Injury

It was soon noticed in the experiments that all plants of a species in which floral abscission occurs throw off the remains of the pedicel when this organ is severed at any point distal to the separation layer. If after such an operation no abscission occurs, it can be safely concluded that floral abscission never occurs in that species. *Petunia hybrida, Salpiglossis sinuata, Salpichrora rhomboidea,* and *Lycium australis* are the only species of the list in table 6 which do not abscise flowerless pedicels in this way. *Nicotiana Bigelovii, N. quadrivalvis,* and *N. multivalvis* occasionally do not throw off pedicels under such conditions. The reaction time in cases where the last three species do abscise severed pedicels is very slow (four to fourteen days).

Turning now to the relation of these observations to the induction of abscission by mechanical injury, it is first necessary to recall the controls used in Experiments 5 and 6 (cf. pages 399 and 400). A further consideration of the reaction of these controls will suggest that mechanical injury can induce abscission by the action of the stimulus directly on the cells in the vicinity of the separation zone. The control used in Experiment 5, it will be remembered, showed that abscission does not occur under normal conditions in a series of flowers cut as in figure 9. From the control used in Experiment 6 it is evident that merely cutting off the flower at varying distances from the separation layer, forming pieces as represented in figure 10, causes abscission to occur, evidently as the result of no other stimulus than that of severing the pedicel. Now, if the cut be made through the pedicel at a point approximately 1 mm. distal to the separation layer in flowers, as represented on the extreme right of figure 9, abscission will occur in the remaining piece, which is now scarcely 2 mm. in length. It is evident that the stimulus caused by severing the pedicel must act directly on the cells in close proximity to the separation zone. Practically the same results are obtained when the transverse cut is made through the base or middle of the calyx. There is no reason to suppose that the stimulus set up by cutting through the flower near the base or middle of the calyx differs in any fashion from that offered by a cut severing only the pedicel.

Several interesting conclusions are brought out by an examination of the above facts. In the first place, the abscission of the remains of severed pedicels is probably independent of the transportation of materials from the rest of the plant to the separation zone. It may result from the action of the stimulus directly on the cells in the vicinity of the separation layer and is, therefore, largely independent of such physiological processes as transpiration which might conceivably enter in. In the second place, abscission induced by mechanical injury is probably of the same nature as that of severed pedicels and therefore probably results from the action of the stimulus directly on the cells in immediate proximity to the separation layer.

SUMMARY

The final summary of results given below is presented under several headings corresponding to those of the main body of the paper. Unless otherwise stated, the results given may be taken as applying to all the species of the Solanaceae in which abscission was found to occur. First is presented a complete list of the species which were investigated, indicating by 1 those in which floral abscission never occurs, by 2 those in which it very seldom occurs, and by 3 those which were actually examined microscopically to determine the histological structure of the separation zone and the method of abscission.

3 N. Tabacum var. macrophylla
3 N. sylvestris
3 N. Tabacum "Maryland"
3 F_1H154 (N. sylvestris × N. Tab. var. macrophylla)
3 F_1H179 (N. sylvestris × N. Tabacum "Cuba")
3 F_1H36 (N. sylvestris × N. Tab. var. angustifolia)
N. glauca
3 N. rustica (2 varieties—not brasilia)
2, 3 N. Bigelovii (3 varieties)
2 N. quadrivalvis (2 varieties)
2 N. multivalvis
N. Sanderae
N. rustica var. brasilia
N. suaveolens
3 Solanum umbelliferum
S. tuberosum
S. jasminioides
3 S. verbascifolium
S. nigrum
2, 3 Iochroma tuberosa
3 Cestrum fasciculatum
Lycopersicum esculentum var. vulgare
3 L. esculentum var. pyriforme
1, 3 Petunia hybrida
1, 3 Salpiglossis sinuata
3 Datura sanguineum
1 Salpichrora rhomboidea
1 Lycium australis

Histology and Cytology of the Pedicel

1. The separation layer arises in all the species listed above, except *Lycopersicum* and *Solanum tuberosum,* at or near the base of the pedicel. In the latter two species the layer is located near the middle of the pedicel, but even in these cases, if one considers the pedicel to be composed of two internodes, the layer occurs at the base of the most distal internode.

2. The separation layer is preformed, ready to function at any stage in the development of the flower and represents (cf. Kubart's first type, page 350) a portion of the primary meristem which has retained some of its originally active condition.

3. In all the species except *Datura* the separation cells are characterized by their small size, isodiametric shape, large amount of protoplasm and somewhat collenchymatous appearance. A study of the early histological development of the pedicel indicates that the small size of the separation cells does not necessarily bear any relation to abscission. This statement is supported by the fact that in *Datura* there is absolutely no visible difference between the separation cells and any other cells of the pedicel.

4. Various tests with stains, acids, and alkalis fail to indicate any chemical difference between the cell walls of the separation cells and the walls of neighboring cortical cells which do not separate. However, the middle lamellae of cell walls in the general region at the base of the pedicel seem somewhat more easily hydrolysed by acids than in the more distal portions.

5. A study of the early histological development of the pedicel in *Nicotiana* and *Lycopersicum* shows that the grooves near which the separation zone arises do not necessarily bear any relation to abscission. The grooves are formed because, in the development of the pedicel, certain cells do not increase in size so fast as the neighboring cells on either the proximal or distal side.

6. The development of mechanical tissue in the pedicel of *Nicotiana* continues through the separation layer, thus frequently holding the fruit on the plant in spite of the fact that abscission commonly occurs in the cortex. In most of the berry-forming species of the Solanaceae this mechanical tissue does not become continuous through the separation layer and thus offers no impediment to fall when abscission occurs in that region.

The Process of Abscission

1. The process of abscission conforms to the usual type, which involves the separation of cells along the plane of the middle lamella of the cell wall separating them.

2. No cell divisions or elongations were observed to accompany abscission.

3. All the cells across the pedicel in the region of the separation layer take part in separation except the tracheæ and cuticle, which must be broken mechanically. The total number of cells which may be involved is greater in some species than in others. This number may also vary in the same species because of changes in the external conditions.

4. Cell separation is brought about by the hydrolysis and consequent dissolution of the middle lamella (primary cell membrane) or perhaps both the primary and, in part, secondary cell membranes. The agency active in the hydrolysis of the cell membranes is probably an enzyme.

5. An increase in cell turgor frequently occurs during abscission, but probably serves merely to hasten and facilitate the process. Most of the frequently observed expansion and the turgid appearance of the separation cells during abscission are probably due to the natural release of pressure caused by the dissolution of the middle lamellae.

6: Abscission of the style and corolla in *Nicotiana* and *Datura* resembles, to a large extent, abscission of the flower.

Time of Abscission

1. The length of time between anthesis and normal flower-fall due to lack of fertilization differs among the varieties of *Nicotiana*. This variation was found to range between an average of five to eighteen days in some fifteen species and varieties of *Nicotiana*. A much smaller range of variation (0.7 to four days, with the largest frequency in the three day group) was noted for the time between anthesis and fall of the corolla after pollination.

2. The stimulation of the stylar tissues by the growth of the pollen tubes tends to shorten the time between anthesis and fall of the corolla, this effect being independent of fertilization. Such stimulation of the stylar tissues has no appreciable effect upon floral abscission.

3. Floral abscission occurs in F_1 H179 seven hours after subjecting shoots of the plant to 1.5 per cent illuminating gas at a temperature

of 19° C. It occurs in *Nicotiana Tabacum* "Maryland" in eight hours under the same conditions. The actual time involved in the process of cell separation in the above-mentioned cases lies within thirty to forty minutes in the hybrid and within forty-five to sixty minutes in the *Tabacum* variety. Normal abscission in these forms is much slower

4. The length of the reaction time in cases of flower-fall due to mechanical injury shows that this length of time depends more on the age of the flower than on the type of injury.

5. Temperature is the most important conditioning factor in estimates of the time of abscission.

Experimental Induction of Abscission

1. Floral abscission is induced, in a large number of the species investigated, by illuminating gas or laboratory air. The increase in resistance to abscission stimulated in this manner takes place suddenly in some species, since abscission will not occur after the opening of the corolla. In other species this condition does not exist.

2. It is possible to induce the process of abscission with illuminating gas in small isolated pieces of the pedicels or in longitudinal sections of the pedicel cut free-hand from fresh material.

3. Abscission in *Nicotiana* and *Lycopersicum* is induced by certain types of severe injury and not by others. Injury to the ovary seems more effective in causing abscission than injury to other parts of the flower. In the case of these other flower parts, it seems necessary that a certain amount of tissue be actually removed or destroyed before fall occurs. Injury to the pedicel does not cause abscission unless it breaks entirely the connection between floral organs and stem. Flower-fall in *Lycopersicum* is not readily induced by injury. Floral abscission in this genus is more dependent upon physiological conditions brought on by abnormal soil conditions.

4. Experiments on the induction of abscission in small isolated pieces and in flowers with only a small portion of the stem proximal to the separation layer attached indicate that the stimulus produced by the action of external factors such as illuminating gas and mechanical injury can cause abscission by acting directly on the cells in close proximity to the separation zone. The action of external factors is thus largely independent of such physiological processes as transpiration which might enter in. This statement is supported by experiments which show that abscission is not necessarily induced by checking transpiration from the flower.

CONCLUSION

It is proposed in what follows to take up consideration of such phenomena in connection with abscission as are still but slightly understood. One of the most perplexing of these is undoubtedly the definitely predetermined location of the separation layer when no morphological and sometimes no physiological (*Datura*) difference can be detected between the cells that separate and those that do not. There need be no doubt, however, that such a difference does exist and that a sufficient refinement of technique will serve to detect it.

In considering this matter further it may be recalled that the separation layer in axial abscission is located at or near the base of an internode. There is undoubtedly some connection between this fact and the fact that the cells most active physiologically are often found in this region. The growth of an internode may be brought about by the action of an intercalary meristem located at the base of the organ and a meristem so located in some cases retains its original activity in the mature internode. Now it is well known that the walls of young active cells are more readily subject to hydrolysis than the walls of older cells, because of the fact that the former contain more water. If we assume, then, that the internode is a metabolic gradient with the most active cells at the base, it would be expected that the walls of these cells would be more subject to hydrolysis than any other cells of the internode. If some hydrolysing agency becomes active throughout the pedicel, it might be expected that the walls of the cells at the base of the internode would react first, causing their separation and thus cutting off the flower or internode. By assuming in this way that separation always takes place through the most active cells of the internode it seems possible to explain the predetermined location of the separation layer.

There is undoubtedly some connection between the above problem and the fact that some plants must perfect a separation layer before detachment can take place. In such cases the tissues at the base of the organ are too old for separation. The same stimulus which causes abscission in some species causes a renewal of activity at the basal region of an organ, resulting in cell divisions and new cells. These new cells may, under a continuation of the stimulus, separate one from another.

Another perplexing problem, which also includes many subsidiary problems, relates to the exact course taken by the stimuli in causing

abscission. Experiments described in the present paper have indicated that this course may be direct as well as indirect. Assuming for the present that some of the factors bringing about abscission always act directly while others act indirectly, we might classify the general factors operative in the case of the Solanaceae as follows:

Direct	1. Narcotic vapors.
	2. Injury to floral organs.
	3. Sudden rise in temperature.
	4. Lack of fertilization.
Indirect	5. Changes in soil conditions.
	6. Factors evident in normal physiological development.

The direct factors act directly on the cells at the base of the pedicel and consequently the reaction time must be comparatively rapid. The indirect factors act indirectly through the general physiological condition, which in turn furnishes the direct stimulus for cell separation. In the latter case the reaction time must, as a general rule, be slow. The nature of factors under 6 are most difficult to understand. An example of the action of these factors would be given in those cases where most of the flowers of an inflorescence are normally abscissed leaving only one or two to continue development, and in those species which absciss male flowers after anthesis.

A further analysis of the course of the abscission reaction introduces another unsettled problem—the nature of the agency which is directly responsible for the dissolution of the middle lamella. It has been pointed out before that an enzymatic body of some kind is probably involved. The following discussion brings out certain facts which it is necessary to take into consideration when speculating as to the nature of this supposed enzyme. The activity of the enzymatic body must be subject to both internal and external conditions. The enzymatic material must also be extremely sensitive to slight changes in the normal environment. It must be continually present in the cells of the separation zone and ready at any moment to react to such changes in the environment. A comparison of several species in regard to their abscission reactions to the factors listed above indicates that this supposed enzyme must be more sensitive in some species than in others. Indeed, in certain species in which no abscission occurs the enzyme must be absent from the region of the separation zone or entirely inactive. Finally, it seems necessary to assume that in certain species the action of the enzyme is suddenly inhibited at about the time of the opening of the corolla.

It has been noticed in all the experiments detailed above that older flowers are less subject to "spontaneous" abscission than younger ones. The transition line as to size or age beyond which no abscission occurs can not in most cases be definitely drawn; that is to say, the development of a resistance to stimuli takes place gradually. This is probably explained by the fact that cell walls gradually become less subject to hydrolysis with age. The celluloses and pectoses lose water with age and it is well known that these compounds are subject to hydrolysis in proportion to the amount of water they contain. In those cases where the increase in resistance to stimuli takes place suddenly it is necessary, as suggested above, to assume some kind of inhibitor of the enzymatic action.

The effect that pollination has in hastening abscission of the corolla is a subject which is related to the phenomena described by Fitting (1909) for orchids. The phenomena are as yet only slightly understood. The explanation seems to involve some relaying of stimulus from cell to cell. This is also involved in the explanation of floral abscission induced by injury to the ovary. These two cases and others indicate that in some instances, at least, abscission responses are related to tropistic responses as Fitting (1911) has suggested.

Finally, attention may be called to the fact that the most pressing need in connection with all the problems mentioned above is, in the first place, to establish by some experimental means a definite connection between some enzymatic body and the process of abscission and, in the second place, more definite knowledge as to the rôle which cell turgor plays in cell separation. Taking all the facts into consideration, it is evident that abscission is fundamentally a physiological problem, the crux of which lies, as in all such problems, in the biochemistry of the cell.

The studies reported upon above were carried on under the direction and supervision of Professor T. H. Goodspeed and I am under deep obligation to Professor F. E. Lloyd for many valuable suggestions both throughout the course of the experiments and during the preparation of this report of them.

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PLATE 49

Fig. 1. Base of pedicel of *Nicotiana* bud showing groove, separation zone, and process of abscission well under way in dorsal cortex.

Fig. 2. Portion of cortex in the separation layer of *Nicotiana* showing the bulging of the epidermis, one of the first signs of abscission.

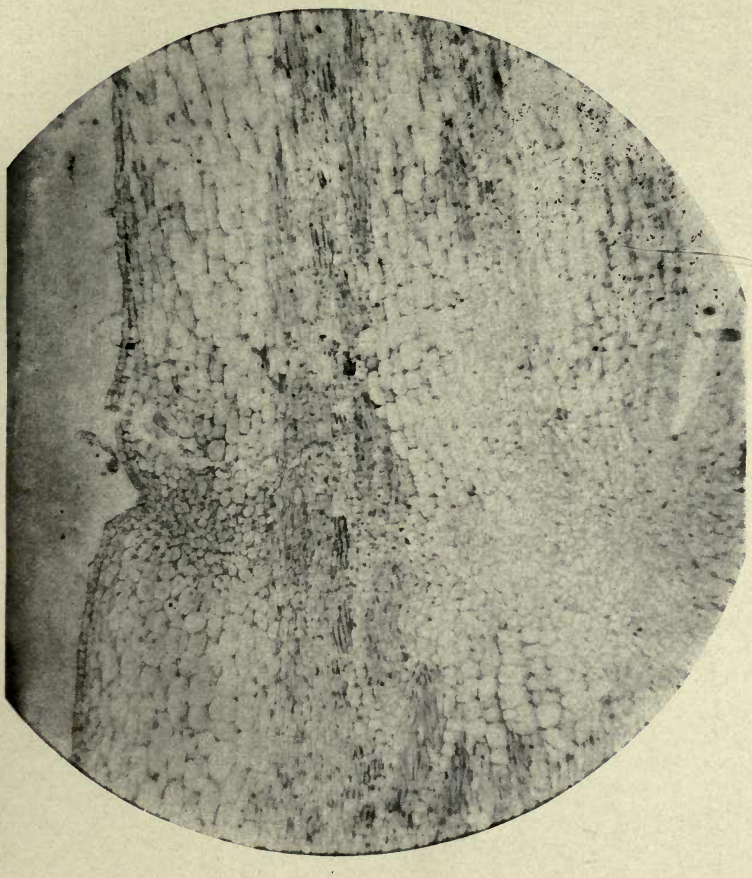


Fig. 1

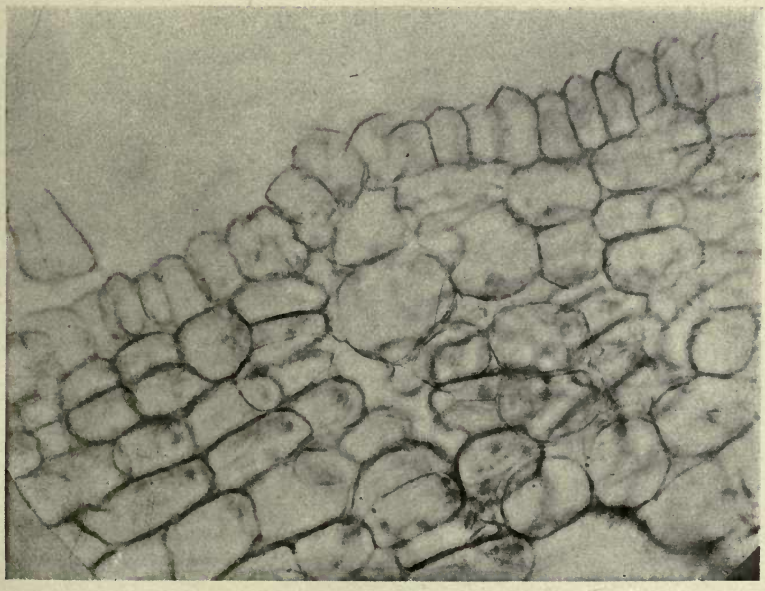


Fig. 2

PLATE 50

Fig. 1. Portion of the base of the pedicel of *Nicotiana* at a late stage in the process of abscission showing the independent origin of the process in the pith.

Fig. 2. Portion of the cortex in the separation layer of *Nicotiana* showing separating cells next to the vascular system.

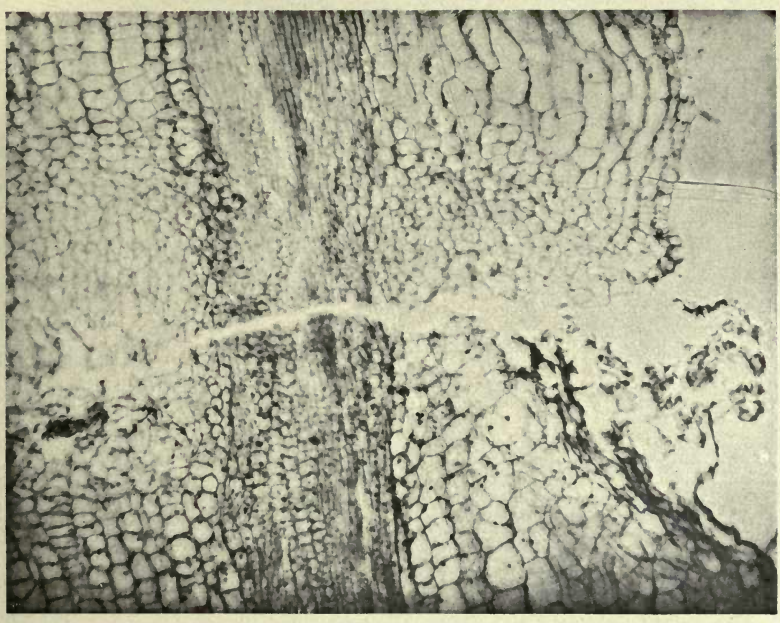


Fig. 1

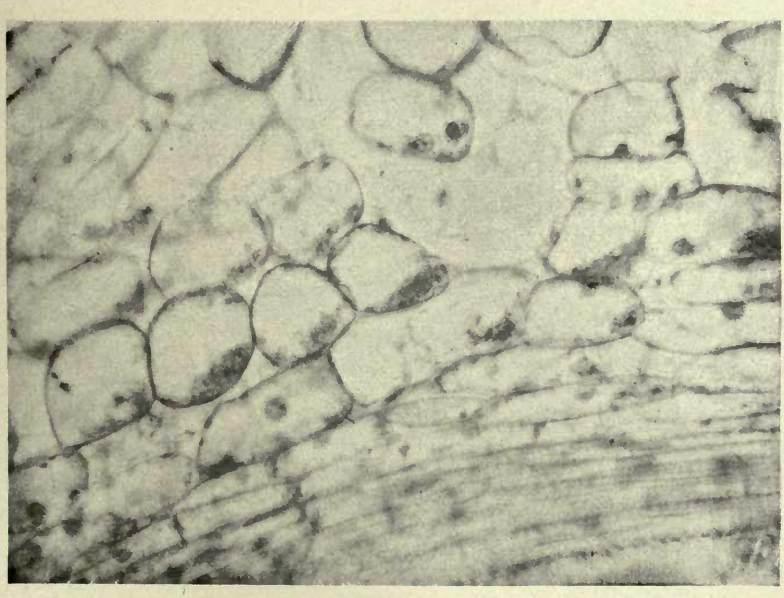


Fig. 2

PLATE 51

Portion of the separation layer of *Nicotiana* showing cells in the process of separation in the upper part of the section.

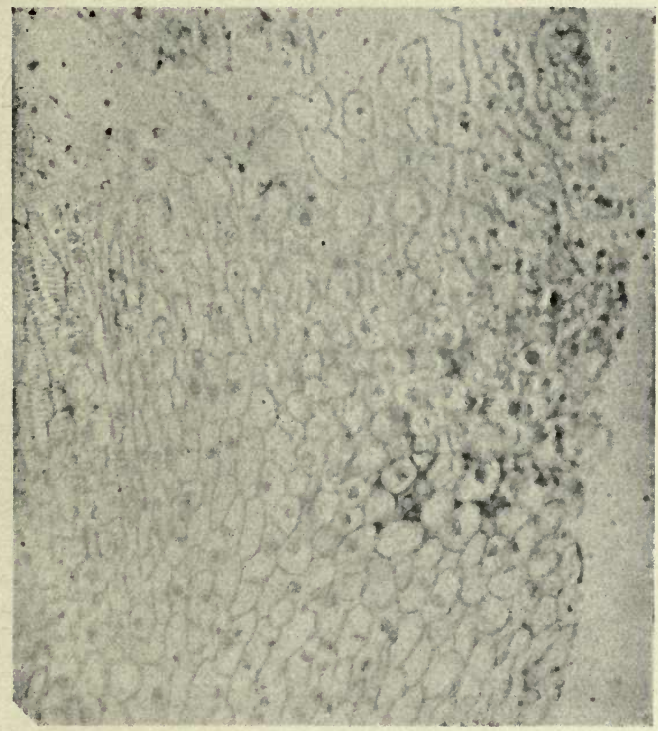
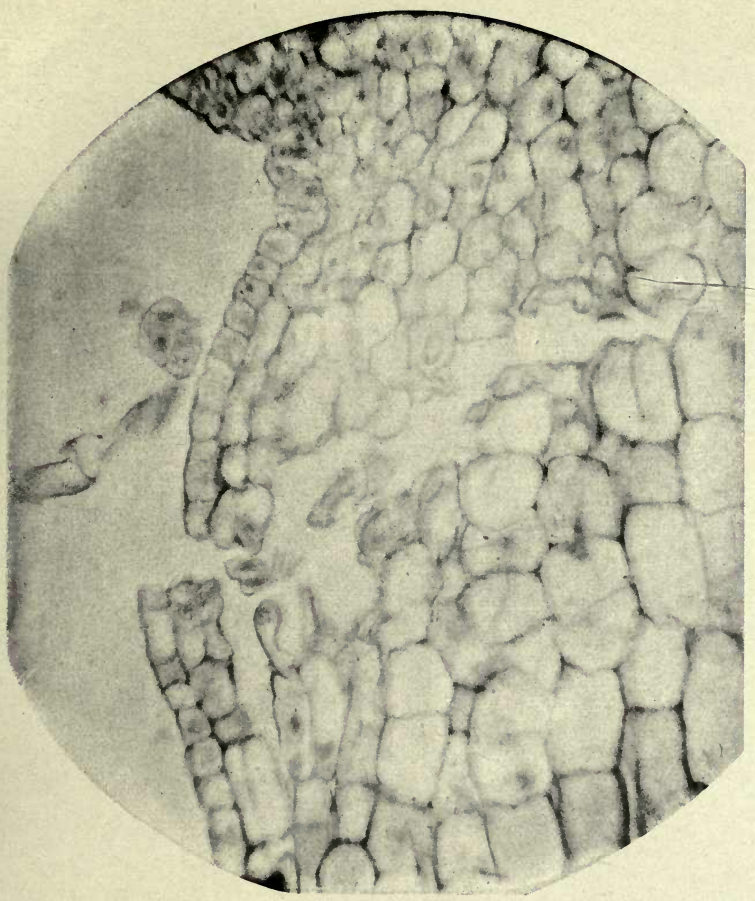


PLATE 52

Fig. 1. Portion of dorsal cortex near the groove in the pedicel of *Nicotiana*, showing the abscission process well under way.

Fig. 2. Group of isolated cells washed off from end of a freshly abscissed pedicel of *Nicotiana*.

Fig. 3. Single isolated cell showing the thinness of the remaining cell membrane.



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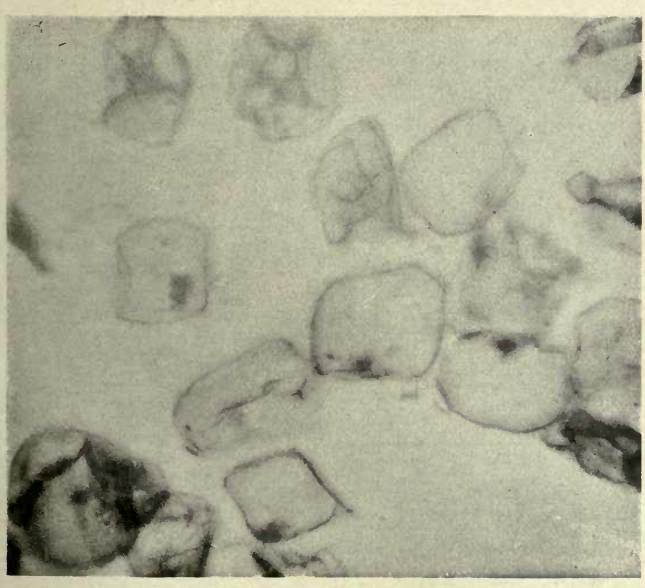

Fig. 2

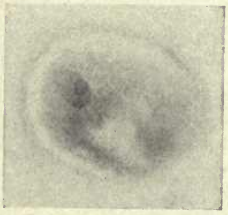

Fig. 3

PLATE 53

Fig. 1. Portion of pedicel of *Lycopersicum*, showing groove and separation zone.

Fig. 2. Portion of cortex of pedicel of *Lycopersicum*, showing groove and abscission process fairly well along; cell separation first takes place between only two tiers of cells before spreading to others.

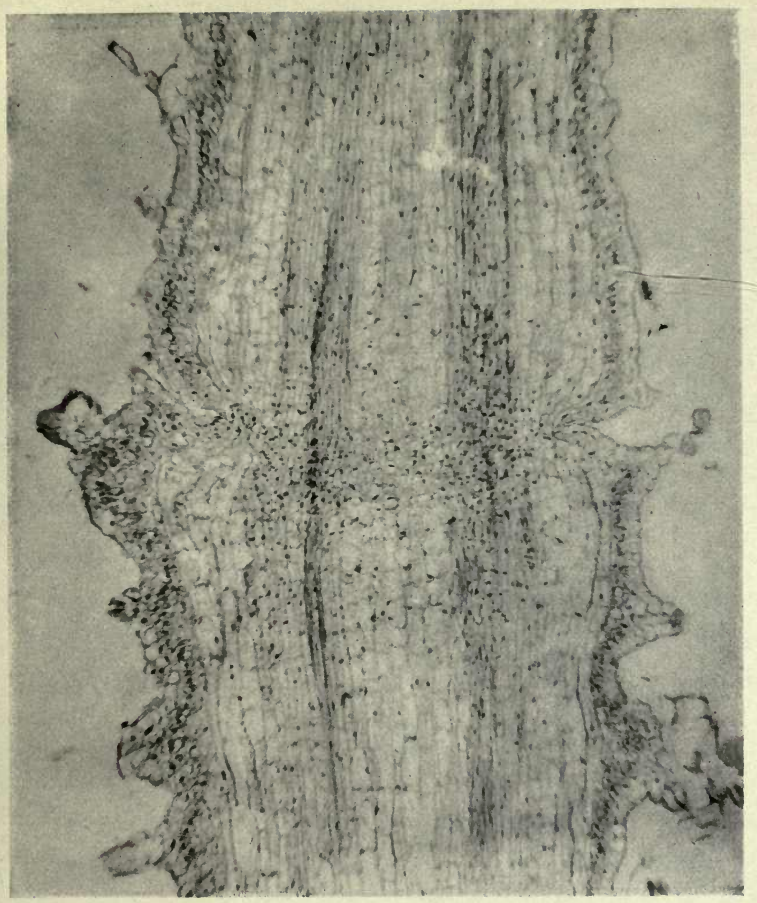

Fig. 1

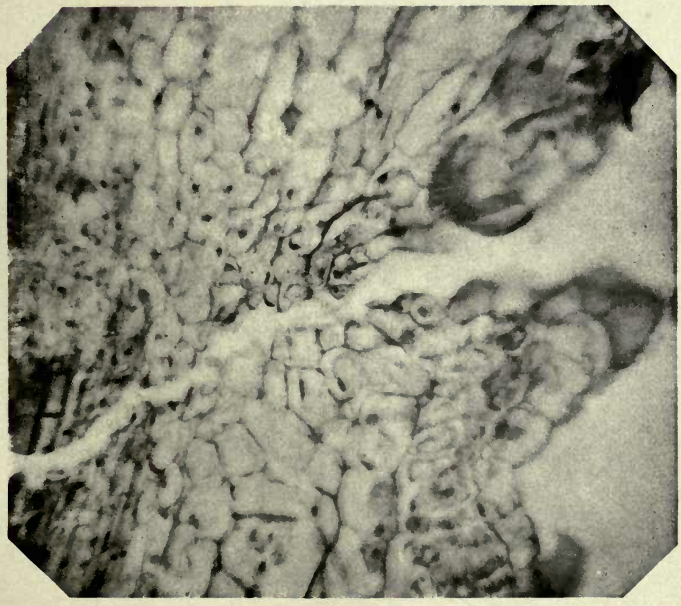

Fig. 2

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